GILEAD SCIENCES

FDA Advisory Committee Briefing Document

VireadÔ
(Tenofovir DF)

**For the Treatment of HIV-1 Infection in Adults
in Combination with Other Antiretroviral Agents**

NDA 21-356

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1.   SUMMARY

Despite improvements in morbidity and mortality, a substantial number of patients do not achieve adequate suppression of HIV viral replication with available antiretroviral therapies. Drug intolerance, inadequate adherence to the prescribed regimen, pharmacokinetic or pharmacodynamic drug interactions, and the emergence of resistant strains have each been implicated as reasons that patients either fail to achieve or experience a loss of virologic control. New therapeutic options are needed that provide durable anti-HIV activity, are well-tolerated, and contribute to improvements in the quality of life for patients infected with HIV. In particular, there is a need for new therapies for patients who have failed multiple regimens. While recognizing the difficulties associated with the development of drugs for this segment of the patient population, community representatives, their caregivers, and regulatory authorities, including the Division of Antiviral Drugs have encouraged sponsors to include treatment-experienced patients in their drug development programs.

Tenofovir is a novel nucleotide analog with activity against human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) and hepatitis B virus. Tenofovir diphosphate (PMPApp), the active intracellular moiety, is a potent inhibitor of retroviral reverse transcriptase and acts as a DNA chain terminator.

During preclinical evaluation, tenofovir demonstrated low oral bioavailability. An orally available prodrug of tenofovir, tenofovir disoproxil fumarate (DF), was selected for clinical development because of its advantageous pharmacokinetic profile, oral bioavailability, potent antiviral activity, and unique in vitro resistance profile.

Clinical development of tenofovir DF was initiated in 1997, leading to submission of a New Drug Application (NDA) in May 2001. Tenofovir DF was submitted for accelerated approval based on surrogate efficacy as well as safety endpoints from 24-week data; along with safety data from extended dosing phases of the registrational studies, wherein patients have been treated for a mean of 58 weeks with some patients having received tenofovir DF for up to 143 weeks. The application is based on the overall evidence accumulated in adequate and well-controlled studies demonstrating the efficacy and safety of tenofovir DF for treatment-experienced HIV-infected patients

The NDA for tenofovir DF includes data from nearly 1,050 HIV-infected patients who have been enrolled and treated in clinical studies evaluating safety, pharmacokinetics, and antiviral activity. Clinical studies have been complemented by extensive genotypic and phenotypic assessment of clinical HIV isolates. The results demonstrate that tenofovir DF taken once daily has an excellent safety profile and significant anti-HIV activity, including activity against multi-drug resistant HIV.

Pivotal clinical studies reported in the NDA focused on HIV-infected adults receiving various combinations of the available anti-HIV drugs (studies 902 and 907). Additional studies evaluated pharmacokinetics and bioequivalence (studies 901, 909, and 914) and

safety (study 908). A 96-week study (study 903) designed to support traditional approval is currently underway to evaluate the safety and efficacy of tenofovir DF in treatment-naïve patients. Results from the initial 48 weeks of study 903 will be available in Spring 2002. A second confirmatory study (study 928) will be conducted in treatment-experienced pediatric patients.

Expanded access programs are ongoing in several countries and have enrolled 3,880 patients as of 27 August 2001, including 2,214 in the U.S.

2.   INTRODUCTION

Tenofovir disoproxil fumarate (9-[(R)-2-[[bis[[(isopropoxycarbonyl)oxy] methoxy]phosphinyl]methoxy]propyl] adenine fumarate 1:1,tenofovir DF) is an orally bioavailable ester prodrug of tenofovir (also known as PMPA), an acyclic nucleotide analog with activity in vitro against retroviruses, including HIV-1, HIV-2, and hepatitis B virus (HBV). Due to the presence of a phosphonate group, tenofovir is negatively charged at neutral pH, which limits its oral bioavailability. Following absorption, tenofovir DF is rapidly converted to tenofovir which is metabolized intracellularly to the active metabolite, tenofovir diphosphate, a competitive inhibitor of HIV-1 reverse transcriptase (RT) that terminates the growing DNA chain.

Acyclic phosphonomethylether nucleosides like tenofovir exhibit distinct biological properties. Due to their efficient intracellular activation to their active metabolites, they possess potent antiviral activity in vivo. Also, the parent compounds and their metabolites have a prolonged intracellular half-life, which allows for infrequent administration. Finally, induction of antiretroviral resistance to these compounds in vitro has been difficult, possibly as a result of their minimal structure and similarity to the natural substrate (dATP).

Rationale for Development of Tenofovir DF

Despite the availability of potent antiretroviral agents, some patients do not achieve or are unable to maintain a viral load below the limit of assays used in clinical practice (i.e., plasma HIV-1 RNA levels < 400 or < 50 copies/mL). It is likely that many of these virological failures are due to the development of drug resistance or to re-emergence of HIV-1 with pre-existing resistance mutations. Incomplete suppression of virus allows continued replication, particularly of strains with reduced sensitivity to the antiretroviral agents being used, thus putting these patients at increased risk for disease progression or death.[1, 2] Since the development of resistance to the NRTI, NNRTI, and PI components of anti-HIV-1 regimens is a significant problem in achieving long-term, sustained viral suppression, antiretroviral agents that are less prone to resistance development and that maintain activity in the presence of pre-existing mutations are urgently needed. Clinical data in both treatment-naïve and treatment-experienced HIV-1-infected patients demonstrate that tenofovir DF has a unique HIV resistance profile that will satisfy these requirements. In particular, tenofovir DF exhibits activity against viruses displaying mutations with reduced sensitivity to other nucleoside analogs and only rarely selects for virus mutations.

In addition to its unique resistance profile, tenofovir DF meets other desirable requirements of a new antiretroviral agent. Tenofovir DF is a potent agent that is synergistic or additive when combined with all antiretroviral agents inhibiting the same or different aspects of viral replication. Tenofovir DF is administered on a once daily dosing schedule, an important requirement for simplifying treatment regimens and improving adherence. The long intracellular half-life means that delay in dosing would prevent a significant fall in drug level and rebound in viral replication. Finally, no evidence of clinically significant tenofovir DF-

related toxicity has been demonstrated in treatment-experienced and treatment-naïve HIV-infected patients.

Overview of Clinical Development Program

Gilead Sciences has undertaken a clinical program to investigate the antiviral activity of tenofovir DF, either as monotherapy or in combination with a variety of other antiretroviral agents, in antiretroviral treatment-naive and treatment-experienced HIV-infected patients.

The primary focus of the NDA submission and Safety Update is on the data from four clinical studies of oral tenofovir DF in HIV-infected patients: studies 901, 902, 907, and 908. These studies involve approximately 1,050 patients who received tenofovir DF alone (study 901) or in combination with other antiretroviral agents (studies 902, 907, 908, and 910). Pharmacokinetic data are also presented from two studies in healthy volunteers (studies 909 and 914). As background, data are also included from a phase 1 study of intravenous tenofovir (study 701).

Table 2-1 summarizes the completed and ongoing studies in the tenofovir DF development program.

Table 2-1.   **Summary of Tenofovir/Tenofovir DF Clinical Development Program**

Study Number	Patient Population	Initial Dose (s) (mg per day)	Enrolled /Target	Location	Status (as of 01 August 2001)
Phase 1 / 2 Studies in HIV-Infected Patients					
701	Naïve and Experienced	1 mg/kg, 3 mg/kg (IV)*	20/40	US	Complete
901	Naïve and Experienced	75, 150, 300, 600 mg	59/50	US	Complete**
917	Naive	300 mg	8/18	US	Enrollment ongoing
Phase 2 / 3 Studies in HIV-Infected Patients					
902	Experienced	75, 150, 300 mg	189/175	US	Complete**
907	Experienced	300 mg	552/600	US, Europe, Australia	Complete**
903	Naïve	300 mg	601/600	US. Europe, S. America	Enrollment complete; study ongoing.
Open-Label Safety Studies					
908	Experienced	300 mg	296/300	US	Enrollment complete study ongoing.
910**	Experienced	300 mg	335/614	US. Europe	Study ongoing.
Phase 1 Pharmacokinetic Studies in Healthy Volunteers					
909	Healthy Volunteers	300 mg	103/93	US	Complete
914	Healthy Volunteers	300 mg	40/40	US	Complete
Expanded Access Program					
Various	Experienced	300 mg	Not applicable	US, Europe, and Canada, Australia	Enrollment ongoing

*   Study 701 involved tenofovir given intravenously
** A rollover protocol (study 910) was made available in October 2000 for patients in studies 901, 902, and 907
    remaining on tenofovir DF beyond 48 weeks of treatment.

3. PRECLINICAL SUMMARY

The antiviral activity of tenofovir has been demonstrated using in vitro assays and in vivo animal models, including SIV infected macaques. Toxicology studies were conducted in rats and dogs with tenofovir administered by the intravenous (iv) route to support initial clinical studies in which tenofovir was administered iv. The major target organs of toxicity identified in these short term studies were kidney, gastrointestinal tract, and bone.

The definitive preclinical ADME and toxicology studies for this compound were conducted using tenofovir DF administered orally. The bioavailability and pharmacokinetic profile of tenofovir DF have been studied in mice, rats, dogs and rhesus monkeys. Acute, subchronic and/or chronic toxicology studies of tenofovir DF have been conducted in mice, rats and dogs. Rhesus monkeys have been used to further define the acute and subchronic toxicologic effects of tenofovir DF. Long-term carcinogenicity studies in rats and mice are in progress.

3.1. Mechanism of Action

Tenofovir is taken up by cells and undergoes phosphorylation to the antivirally active metabolite PMPApp.[3] PMPApp competitively inhibits both RNA- and DNA-directed reverse transcriptase activity. PMPApp competes with deoxyadenosine triphosphate (dATP) for incorporation into nascent DNA and, since it lacks a 3' hydroxyl group, causes premature chain termination. The K_i for reverse transcription (RNA-directed DNA synthesis) is 0.02 µM, more than 200-fold lower than the K_i for human DNA polymerase α, and more than 3,000-fold lower than the K_i values for β and γ. Generally, the lowest incorporation efficiencies with all three polymerases (α, β, and γ) were found for PMPApp when compared to ddATP, ddCTP, 3TCTP, d4TTP, or PMEGpp.[4]

3.2. In Vitro Cytoxicity

The cytotoxicity of tenofovir was determined both in quiescent and activated peripheral blood mononuclear cells (PBMCs) and in an established T-lymphocytic MT-2 cell line. In PBMCs and MT-2 cells, tenofovir exhibited low cytotoxicity with CC_{50} values > 1 mM. In quiescent PBMCs, no cytotoxic effect of tenofovir was detected at concentrations as high as 100 µM[5]

3.3. Preclinical Evaluation of Antiviral Activity

Tenofovir and tenofovir DF were evaluated in vitro for antiviral activity (IC_{50}) and cytotoxicity (CC_{50}) in HIV-1 IIIb and clinical strain 96-250 in MT-2 cells, peripheral blood mononuclear cells (PBMCs), or a macrophage/dendritic cell coculture. The selectivity index ($SI = CC_{50}/IC_{50}$) for tenofovir was > 2,000. Due to its increased cellular permeability, the anti-HIV activity of tenofovir DF is increased by 17- to 90-fold over tenofovir.[5]

The concentration of tenofovir required for 50% inhibition (IC_{50}) of wild-type HIV-1_{IIIB} is 1-6 µM in MT-2 or MT-4 cells (based on inhibition of viral cytopathic effect) and 0.2-0.6 µM in PBMCs (based on inhibition of virus production).[6-8] The activity of tenofovir against clinical HIV-1 isolates from non-B subtypes has also been studied.[9] The mean IC_{50} values for tenofovir against HIV-1 subtypes A, C, D, E, F, G, and O in primary PBMC cultures were all within twofold of the subtype B IC_{50} value (range: 0.55 to 2.2 µM). Tenofovir has also been shown to be active in vitro against HIV-2, with similar potency as observed against HIV-1 (IC_{50} of 4.9 µM in MT-4 cells).[7]

Tenofovir, paired individually with 14 other antiretroviral compounds, was tested for additive, antagonistic, or synergistic antiviral activity against HIV-1 in MT-2 cells[10, 11] Tenofovir showed minor to moderate synergy with ddI and nelfinavir, and strong synergy with ZDV, amprenavir, and all non-nucleoside reverse transcriptase inhibitors (NNRTIs) tested. The other combinations were additive, and no significant antiviral antagonism was observed. Combinations of tenofovir and 50 µM hydroxyurea show greater than a 26-fold decrease in the tenofovir IC_{50} value for the wild-type HIV molecular clone NL4-3. Notably, HIV isolates with RT mutations associated with slightly decreased susceptibility to tenofovir show hypersusceptibility to tenofovir in the presence of hydroxyurea. However, this synergy was not demonstrated in vivo in study 901.

The in vivo antiviral activity of tenofovir (sc) was demonstrated in murine, feline and primate models. Tenofovir DF (po) and tenofovir (sc) had comparable activity in murine sarcoma virus- (MSV)-infected severe combined immunodeficient mouse model (SCID mice); orally administered tenofovir had no effect, consistent with its poor oral bioavailability).[12, 13] In studies of acute and chronic FIV infection in cats, tenofovir (30 mg/kg/day) reduced circulating viral FIV RNA but not PBMC-associated, co-culture-detected virus burden.[14] In a study of SIV-infected juvenile macaques, tenofovir (30 or 75 mg/kg/day) was more efficacious than zidovudine (100 mg/kg/day) as assessed by surrogate markers of SIV infection and clinical status.[15] In further studies in SIV-infected rhesus macaques, tenofovir DF was effective in preventing infection, reducing viral load and slowing progression of disease, maintaining long term (> 2 year) efficacy, and producing clinical benefit in the presence of partially resistant strains of virus.[16, 17]

3.3.1.   Anti-Hepatitis B (HBV) Activity

Tenofovir is a potent and selective inhibitor of HBV in vitro. Tenofovir inhibits HBV production in HepG2 2.2.15 and HB611 cells with IC_{50} values of 1.1 and 2.5 µM, respectively, and corresponding CC_{50} values of > 100 and 260 µM, respectively.[18, 19] As observed with anti-HIV activity, tenofovir DF showed increased in vitro potency against HBV in comparison with tenofovir. Tenofovir was also shown to be equally effective against both wild-type and lamivudine-resistant HBV in a cell culture assay.[19, 20] In contrast, lamivudine demonstrated > 200-fold reduced activity against this HBV mutant. Tenofovir has also been shown to inhibit the replication of duck HBV (DHBV) in primary duck hepatocytes with an IC_{50} of 0.11 µM.[21]

3.4. Mitochondrial Toxicity

A variety of clinical symptoms observed in HIV patients treated with prolonged NRTI therapy may be linked to mitochondrial toxicity. These include myopathy and cardiomyopathy, polyneuropathy, lactic acidosis, pancreatitis, lipodystrophy and possibly others. Tenofovir DF was compared with nucleoside reverse transcriptase inhibitors (NRTIs) for effects on mitochondrial DNA (mtDNA) synthesis and lactic acid production.

In HepG2 human liver cells, tenofovir DF (3-300 µM), lamivudine and abacavir had no effect on mitochondrial DNA content, zidovudine and stavudine caused 30-40% reduction, and ddC and ddI caused marked depletion of mtDNA.[22] In normal human skeletal muscle cells (proliferating or quiescent), tenofovir DF (300 µM), lamivudine, abacavir and zidovudine had no effect on mtDNA levels, stavudine caused moderate reduction, and ddC and ddI showed marked depletion of mtDNA.[22]

Tenofovir DF and lamivudine did not increase the lactic acid production in HepG2 cells and skeletal muscle cells relative to the untreated control, whereas zidovudine produced a concentration-dependent increase in the lactate production in both cell types tested.[22] Results of these in vitro studies suggest a low potential of tenofovir DF to interfere with mitochondrial functions.

In vivo, no evidence of mitochondrial-related hepatic, hematologic, cardiac, pancreatic, or skeletal muscle toxicity was detected in chronic toxicity studies (42-week) in rats and dogs.[23, 24]

3.5. Nonclinical Pharmacology and Toxicology

The nonclinical safety profile of adefovir dipivoxil has been extensively evaluated in pharmacokinetic/ADME, pharmacology, and toxicology studies using test systems and protocols accepted by the ICH and international health authorities. This evaluation has included studies in mice, rats, guinea pigs, rabbits, dogs, and monkeys. The principal target organs of toxicity following oral administration of tenofovir DF or subcutaneous administration of tenofovir in animal models were the gastrointestinal tract, kidneys and bone. Nephrotoxicity was the primary dose-limiting toxicity associated with the oral administration of tenofovir DF in dogs and monkeys; gastrointestinal toxicity was dose-limiting in rats. Toxicity to bone was demonstrated to be secondary to dose-, species-, and age-related alterations in phosphate homeostasis, resulting from inhibition of intestinal phosphate absorption and/or renal phosphate reabsorption. Tenofovir had no adverse effects on fertility and reproductive performance, embryo-fetal development or peri- and postnatal development. Like marketed nucleoside analogue antivirals, in *in vitro* genetic toxicity studies, tenofovir DF induces chromosomal aberrations but not point mutations. Tenofovir was negative in the *in vivo* mouse micronucleus assay. Carcinogenicity studies are ongoing. In all species examined, tenofovir DF was hydrolyzed to tenofovir following absorption, and tenofovir was cleared exclusively by renal elimination, without further metabolic changes, by a combination of glomerular filtration and tubular secretion.

Conclusions from the nonclinical evaluations are as follows:

- Virology, pharmacology, pharmacokinetics, and toxicology studies provide scientific support for the potential safety and efficacy of tenofovir DF at the proposed human dose.

- The toxicity profile of tenofovir DF has been well characterized in animals and mechanisms of target organ toxicity are generally understood.

- Potential clinical adverse effects are reversible and easily monitored.

3.5.1. Safety Pharmacology

Single oral doses of tenofovir DF had no adverse effects on the central nervous system (male rats, 50 or 500 mg/kg)[25] or on cardiovascular and respiratory function (conscious male dogs, 30 mg/kg).[26] An assessment of effects on renal function demonstrated increased urinary electrolyte excretion and urine volume in rats administered tenofovir DF 500 mg/kg; no effect was observed at 50 mg/kg.[27] When rats were administered tenofovir DF (0, 50, or 500 mg/kg) to evaluate effects on the gastrointestinal transit of a charcoal meal, there was reduced gastric emptying at 500 mg/kg/day, but no effect at 50 mg/kg/day.[28]

3.5.2. Absorption, Distribution, Metabolism, and Excretion (ADME)

Following oral administration of tenofovir DF, maximum tenofovir plasma concentrations were reached within 0.25 to 1.5 hours and declined in a biphasic manner. The observed terminal half-life values were approximately 7, 9, and 60 hours in rats[29-31], monkeys[32] and dogs,[32-35] respectively. Due to the long terminal half-life in dogs, a substantial degree of accumulation was observed upon daily repeat dosing. The oral bioavailability of tenofovir DF was greatest in dogs and monkeys (30-40%) and least in rodents (10-20%). The prodrug moiety was efficiently cleaved in all species such that minimal intact prodrug was observed in systemic circulation. No circulating metabolites of tenofovir, other than the monoester, observed at early time points in rats and dogs, have been observed.[36, 37] This is consistent with the lack of metabolism of tenofovir in intestinal and liver homogenates. A small but statistically significant degree of CYP P450 induction (CYP 1A and 2B) was observed in livers from rats given tenofovir DF at doses of 400 mg/kg/day.[38] Given the known differences in cytochrome P450 across species, the clinical relevance of this observation in humans is unknown.[39] Extensive tissue distribution, suggested by the plasma pharmacokinetics of tenofovir, was confirmed in studies with ^{14}C-labeled tenofovir in dogs[40] and rats.[41] Major sites of tissue uptake included the liver and kidney. Tenofovir was excreted (14-24% of plasma concentrations) but not concentrated in milk from lactating rats.[42] Tenofovir was excreted unchanged in the urine of all animal species tested and renal excretion was identified as the primary route of elimination[36, 43, 44]

3.5.3.   Target Organ Toxicity

The nonclinical toxicity of tenofovir DF was studied in mice,[45, 46] rats,[23, 47-50] rabbits,[51] and dogs.[24, 52] Special studies to elucidate mechanisms of toxicity were conducted primarily in rats and monkeys. The target organs of toxicity identified in the preclinical program were the gastrointestinal tract, renal tubular epithelium, and bone.

3.5.3.1.   Gastrointestinal Toxicity

Gastrointestinal (GI) toxicity, observed primarily in rats, was dose related, reversible, and characterized by inflammation of the stomach and intestines, epithelial cytomegaly in the duodenum and jejunum, and villous atrophy of the ileum in rodents.[23, 53] GI toxicity appeared to be related to high local concentrations in the GI tract of rats reflecting high doses evaluated in toxicology studies (100-1000 mg/kg/day) to compensate for the relatively low oral bioavailability of tenofovir DF in this species. Detection of toxicity to the gastrointestinal tract appeared to be inversely proportional to the length of the tenofovir DF administration period; this may represent an adaptation to the effects of the drug. Acute GI toxicity was observed in dogs administered tenofovir DF 180 mg/kg/day for 5 days;[54] no GI toxicity was observed in dogs administered tenofovir DF chronically (30 mg/kg/day for 42 weeks).[24] No GI toxicity occurred in monkeys administered tenofovir DF for 56 days at doses up to 50 mg/kg/day.[55]

3.5.3.2.   Nephrotoxicity

Renal tubular epithelial karyomegaly, a morphologic change without pathologic consequence, was the most sensitive histological indicator of an effect on the kidney and was observed in rats,[23] dogs,[24] and monkeys.[55] In dogs, the species most sensitive to effects on the kidney, additional microscopic alterations reported following chronic administration of tenofovir DF ($\geq$ 10 mg/kg/day for 42 weeks) included individual cell necrosis, tubular dilatation, degeneration/regeneration, pigment accumulation, and interstitial nephritis. Associated biochemical changes in dogs administered tenofovir DF 30 mg/kg/day were a slight elevation in serum creatinine, glucosuria, proteinuria, and increased urine volume. The incidence and severity of nephrotoxicity was dose related. Effects were reversible following cessation of treatment.[24] In rats administered tenofovir DF 1000 mg/kg/day for 42 weeks, slight elevations in serum creatinine were observed; no biochemical or histopathologic evidence of nephrotoxicity was observed in rat at 300 mg/kg/day. Rhesus monkeys administered tenofovir DF at dose of 250 mg/kg/day or more developed biochemical and/or histopathologic evidence of nephrotoxicity; no nephrotoxicity was observed in monkeys at 30 mg/kg/day.

In vitro models for renal proximal tubular toxicity were used to investigate the in vivo differences in nephrotoxicity observed between the structurally related antiviral nucleotide analogues: tenofovir DF, adefovir dipivoxil (ADV), and cidofovir (CDV).[56, 57] Steady-state transport kinetic experiments in CHO cells stably expressing the functional human renal organic anion transporter 1 (hOAT1) revealed a similar transport efficiency (calculated as

V_{max}/K_m ratio) for the three analogs. Clinical pharmacokinetic data indicate a similar rate of active tubular secretion for the three nucleotides. These observations suggest that a lack of interference with essential intracellular function(s) rather than a difference in renal transport and accumulation in proximal tubule cells is responsible for the improved nephrotoxicity profile of tenofovir DF compared to cidofovir and adefovir.

Tenofovir DF had minimal effect on the growth of primary human renal proximal tubular epithelial cells (RPTECs)(CC50>2 mM), long-term viability of quiescent RPTECs (0.5 mM), and integrity-differentiated proximal tubular epithelium formed by RPTECs (>3 mM). In contrast, CDV (0.5 mM) and ADV (0.3) reduced the half-life of quiescent RPTECs and reduced tubular epithelium integrity (CDV - 0.1 mM; ADV - 1.1 mM).[56, 57] Overall, these in vitro findings correlate with the in vivo nephrotoxicity potential of the three antiviral nucleotides and support the nonclinical and clinical observations of low nephrotoxic potential of tenofovir DF.

3.5.3.3. Bone

Chronic administrations of high doses of tenofovir (sc) or tenofovir DF (po) to immature animals resulted in reversible bone alterations ranging in severity from minimal decreases in bone mineral density and content (oral tenofovir DF: rats and dogs),[23, 24] to pathologic osteomalacia (subcutaneous tenofovir: monkeys).[58-60] Effects were dose/exposure-, age-, and species-specific.

Osteomalacia was reported in juvenile rhesus monkeys administered tenofovir (30 mg/kg/day, sc; AUC = 25X humans at 300 mg/day) chronically in anti-SIV efficacy studies. Marked hypophosphatemia was seen in effected monkeys. Monkeys treated chronically with tenofovir (sc) 10 mg/kg/day (AUC = 8X humans), had no clinical or radiographic evidence of bone toxicity, and animals who were dose-reduced from 30 mg/kg/day to 10 mg/kg/day showed improvement in bone parameters.[60-62]

Based on the findings in the SIV efficacy models, bone morphometry (peripheral quantitative computed tomography, pQCT) and biochemistry evaluations were added to ongoing chronic oral toxicity studies of tenofovir DF in rats and dogs (urinary markers of bone resorption were urinary calcium, deoxypyridinoline in rats and N-telopeptide in dogs; serum markers of bone formation were osteocalcin in rats and sALP in dogs). No gross or microscopic alterations in bone were observed in the chronic toxicity studies. Marginal to slight decreases in bone mineral density and content were seen by pQCT (distal femoral metaphyses and mid-femoral diaphyses) after 13 and 42 weeks of orally administered tenofovir DF in rats (300 - 1,000 mg/kg/day; AUC = 6X humans) and dogs (30 mg/kg/day; AUC = 10X humans). There was a dose related, slight to mild increase in urinary phosphate, marked increase in urinary calcium (high doses). Alterations in biochemical markers were increased deoxypyridinoline (rats) and N-telopeptide (dogs), and increased serum parathyroid hormone, osteocalcin (rats) and bone-alkaline phosphatase (dogs), suggesting increased bone turnover. All bone parameters showed a recovery following a treatment-free period.[23, 24]

In addition to specialized evaluations incorporated into the standard toxicology program, Gilead Sciences undertook specific studies to better delineate the mechanism underlying the effects on bone. These studies suggest that tenofovir is not directly toxic to bone.[49, 51, 58, 63] Instead, data suggest that bone effects are secondary to negative phosphate balance resulting from tenofovir-related reductions in intestinal phosphate absorption and/or renal reabsorption of phosphate.[23, 55, 64-66] Effects of tenofovir on intestinal absorption and renal reabsorption of phosphate appear to be related to inhibition of NaPi transporter proteins.[67] Secondary effects of tenofovir on bone are dose-, species-, and age-related and are reversible with dose-reduction or discontinuation of treatment.

3.5.4.   Genetic and Reproductive Toxicity

The genetic toxicology profile of tenofovir DF is similar to that of marketed nucleoside analogs. Tenofovir DF was negative in the in vitro bacterial mutation (Ames) assay (*Salmonella-Eschericia coli*/ Mammalian-Microsome Reverse Mutation Assay)[68] but positive in the in vitro mouse lymphoma assay (L5178Y TK+/- Forward Mutation Assay), with and without metabolic activation.[69] Tenofovir DF was negative in the in vivo mouse micronucleus assay at plasma exposure levels of more than 10x the human exposure.[70]

Reproductive toxicity was evaluated in rats and rabbits. Tenofovir DF had no adverse effects on fertility or general reproductive performance in rats at doses up to 600 mg/kg/day.[48] Tenofovir DF had no adverse effects on embryo-fetal development in rats[49] at doses 450 mg/kg/day and in rabbits[51] at doses up to 300 mg/kg/day. In a study of effects on peri- and postnatal development in rats, effects considered due to maternal toxicity (450-600 mg/kg/day) were reduced survival and a slight delay in sexual maturation in the F1 generation. There were no adverse effects on growth, development, behavior, or reproductive parameters at non-maternally toxic doses (150 mg/kg/day).[50]

4. CLINICAL SUMMARY

4.1. Efficacy

4.1.1. Introduction

Results from studies 701 (intravenous tenofovir) and 901 (oral tenofovir DF) provided initial confirmation of the antiviral activity of tenofovir and tenofovir DF, respectively. Following 7 daily doses of tenofovir 1 mg/kg or 3 mg/kg, administered intravenously, patients in study 701 had median decreases of 0.58 and 1.05 $\log_{10}$ copies/mL in plasma HIV-1 RNA levels, respectively. For patients who received 28 days of repeat daily dosing with tenofovir DF 300 mg once daily in study 901, the median decrease was 1.22 $\log_{10}$ copies/mL.

In addition to these mixed populations of treatment-naïve and treatment-experienced patients, the effects of tenofovir DF were investigated, in combination with other antiretroviral drugs, in HIV-infected patients whose viral loads were not controlled by their current antiretroviral regimen. Two pivotal placebo-controlled clinical studies (studies 902 and 907) demonstrate the efficacy of tenofovir DF administered in combination with other antiretroviral agents in extensively treatment-experienced HIV-infected patients with a detectable viral load (> 400 copies/mL). Study 902 was a dose-ranging study of three doses of tenofovir DF (75 mg, 150 mg, 300 mg) compared with placebo, and study 907 was a large phase 3 study of tenofovir DF 300 mg compared with placebo. In both studies, an intensification strategy was used in which tenofovir DF was added to existing regimens in a double-blind manner. Significant anti-HIV activity was demonstrated in these antiretroviral-experienced patients despite the fact that 94% of patients in each of the studies had evidence of nucleoside-associated resistance mutations at baseline.

4.1.2. Study 701

The initial clinical evaluation of tenofovir was conducted using an intravenous formulation of the parent compound, tenofovir, administered over one hour. In this placebo-controlled, dose-escalating study, each patient received a total of 8 doses of tenofovir 1 mg/kg, 3 mg/kg or placebo. Dosing occurred on day 1 and on days 8-14; efficacy measures (changes in plasma HIV-1 RNA from baseline and CD4 cell counts) were evaluated periodically through day 42. After 7 days consecutive dosing (days 8-14) patients receiving 1 mg/kg and 3 mg/kg tenofovir had median decreases of 0.58 and 1.05 $\log_{10}$ copies/mL, respectively. In the 3-mg/kg dose group this decrease was sustained through day 21 without additional dosing.

4.1.3. Study 901

Following completion of the development work on an oral prodrug (tenofovir DF) and with confirmation of the antiviral effect of tenofovir in study 701, study 901 was initiated to confirm the pharmacokinetics, anti-HIV activity and safety of the new oral formulation. The design of this study mimicked that of study 701 with an extension of the daily dosing phase

to 28 days. This placebo-controlled, dose-escalating study eventually included 4 doses (75 mg, 150 mg, 300 mg, and 600 mg). One further cohort (75 mg tenofovir DF in combination with hydroxyurea) was studied. After completion of the initial 3 cohorts, an open-label safety phase was added for patients who completed the initial study.

Administration of tenofovir DF once daily for a total of 28 days resulted in statistically significant decreases in HIV-1 RNA levels at all dose levels compared with placebo. In an intent-to-treat analysis of all randomized patients, the median decreases in HIV-1 RNA levels at the end of the 28-day dosing period were -0.33 $\log_{10}$ copies/mL in the 75 mg group, -0.22 $\log_{10}$ copies/mL in the 75 mg + HU group, -0.44 $\log_{10}$ copies/mL in the 150 mg group, -0.85 $\log_{10}$ copies/mL in the 300 mg, and -0.80 $\log_{10}$ copies/mL in the 600 mg group (Figure 4-1).

Figure 4-1.   **Changes From Baseline in Plasma HIV-1 RNA Levels - Study 901, Blinded Phase**
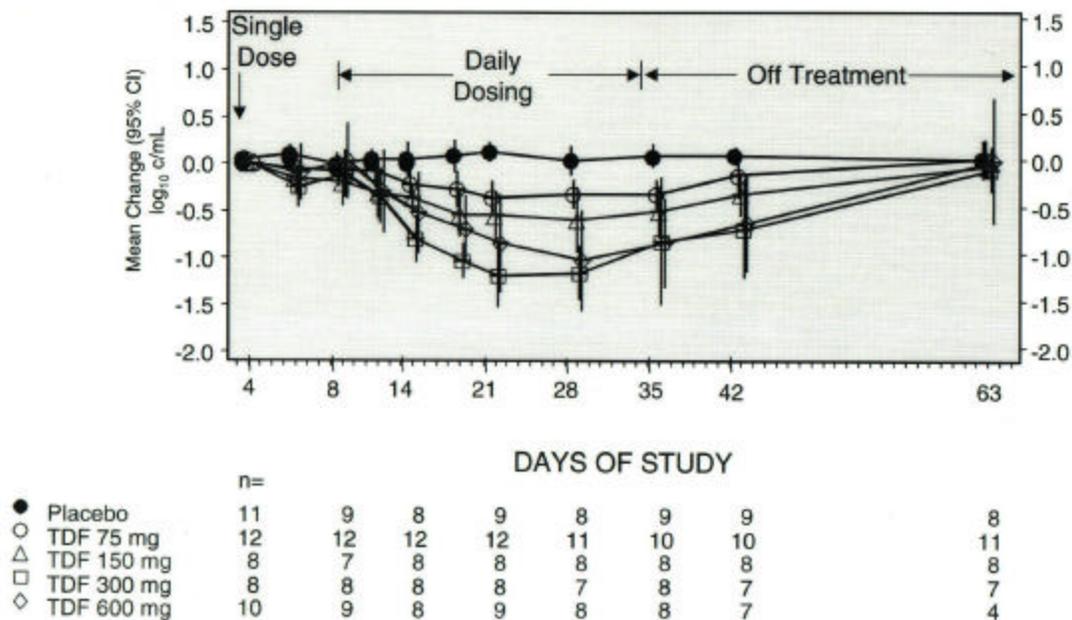
Note: Tenofovir DF 75 mg + HU dose group is not included in graph.

In patients who completed 28 days of dosing, the median decreases in HIV-1 RNA levels were -1.22 and -0.80 $\log_{10}$ copies/mL in those who received tenofovir DF 300 mg and 600 mg, respectively. The median decreases in HIV-1 RNA levels were -1.57 $\log_{10}$ copies/mL and -1.40 $\log_{10}$ copies/mL in patients who had not received previous antiretroviral therapy and -0.97 $\log_{10}$ copies/mL and -0.61 $\log_{10}$ copies/mL in patients who were antiretroviral-experienced in the tenofovir DF 300 mg and 600 mg groups, respectively,

In extended dosing, in combination with highly active retroviral therapy, decreases in plasma HIV-1 RNA levels from baseline were seen at months 6 and 12.

Except for the tenofovir DF 75 mg + HU arm, all dose groups had increases in CD4 counts; however, none of these changes were statistically significant (Table 4-1). In extended dosing, positive changes in CD4 counts were seen at months 6 and 12.

Pharmacokinetic parameters were evaluated in study 901 and are summarized in section 4.4.2.

Table 4-1.   **Changes From Baseline in CD4 Counts (Study 901, Blinded Phase)**

		Tenofovir DF			
CD4 (cells/mm^3)	Placebo	75 mg	150 mg	300 mg	600 mg
Baseline					
N	11	12	8	8	10
Mean	347	447	344	432	330
Median	373	388	274	375	262
Q1 to Q3	259 to 444	278 to 435	253 to 466	323 to 564	185 to 289
Range	164 to 497	214 to 1035	211 to 557	280 to 655	149 to 743
Change at Day 8					
N	9	12	7	8	8
Mean	44	2.1	-24	-17	54
Median	33	9.0	-15	-21	44
Q1 to Q3	7 to 40	-49 to 44	-71 to 21	-34 to -11	15 to 64
Range	-149 to 395	-82 to 101	-95 to 35	-50 to 42	-81 to 268
Change to Day 35					
N	9	10	8	7	8
Mean	74	7	49	7	95
Median	54	42	59	17	64
Q1 to Q3	-22 to 94	-65 to 76	15 to 112	-20 to 38	-8 to 137
Range	-67 to 302	-176 to 98	-103 to 126	-81 to 74	-70 to 443

4.1.4. Study 902

4.1.4.1. Objectives and Study Design

Study 902 was designed to evaluate the long-term (48-week) safety of three doses used in study 901 and to confirm the efficacy results that had been documented. An intensification design was selected to allow characterization of the antiviral activity associated with tenofovir DF in the setting of a stable background antiretroviral regimen. Patients had to be on stable antiretroviral therapy, consisting of no more than four active agents, for at least 8 weeks prior to enrollment.

Between September 1998 and March 1999, 189 patients were randomized in a 2:2:2:1 ratio to add either tenofovir DF at one of three doses (75 mg, 150 mg, or 300 mg once daily) or placebo to their existing regimen in a double-blinded manner. At 24 weeks post-randomization, patients who had received placebo were crossed over to tenofovir DF 300 mg once daily in a blinded fashion for the remainder of the initial 48-week study period (Figure 4-2). Following 48 weeks, patients were offered open-label tenofovir DF 300 mg with continued follow-up. Recently, all patients remaining on this study were rolled over to study 910 for continued long-term follow-up.

Figure 4-2.   Treatment Schedule in Study 902
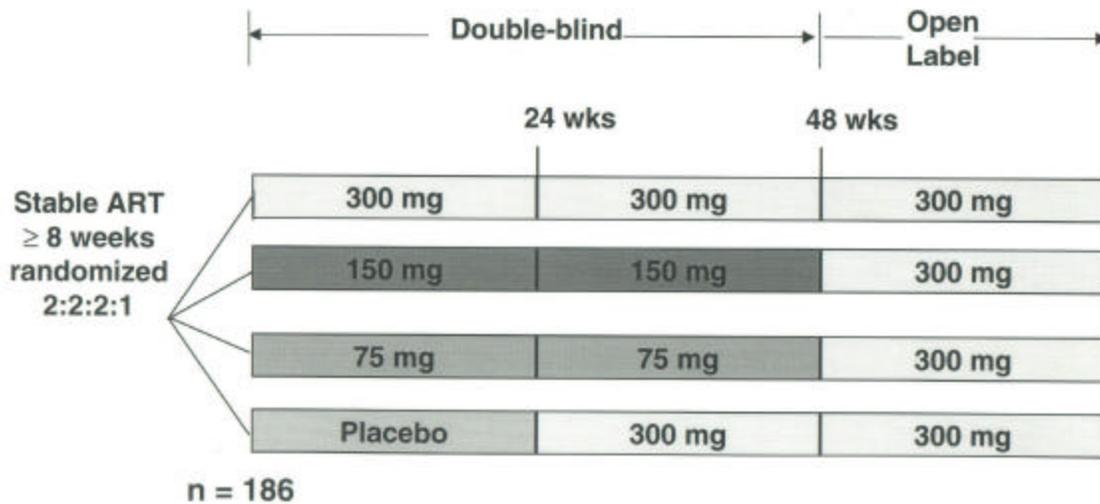
4.1.4.2. Inclusion and Exclusion Criteria

The inclusion and exclusion criteria for study 902 were designed to select a study population that was representative of treatment experienced HIV-infected patients with a detectable viral load. The principal selection criteria was based on plasma HIV-1 RNA levels; ≥ 400 copies/mL and $\leq 100,000$ copies/mL in patients currently receiving stable anti-

retroviral therapy. Other key inclusion criteria were related to the requirements for adequate renal, hepatic and hematologic function. Patients were stratified by site according to HIV-1 RNA level ($\leq$ 5000 copies/mL, > 5,000 copies/mL), CD4 count ($\leq$ 200 cells/mL, > 200 cells/mL), and number of antiretroviral drugs prior to study entry ($\leq$ 4, > 4).

4.1.4.3.   Efficacy Endpoints

The primary and key secondary efficacy endpoints are summarized below.

Primary Efficacy Endpoint

- The time-weighted average change from baseline in $\log_{10}$ copies/mL plasma HIV-1 RNA levels up to week 4 ($DAVG_4$) and to week 24 ($DAVG_{24}$).

Key Secondary Efficacy Endpoints

- The time-weighted average change from baseline in $\log_{10}$ copies/mL HIV-1 RNA levels up to week 48 post-randomization ($DAVG_{48}$).

- The proportion of patients with plasma HIV-1 RNA levels at or below quantification limits ($\leq$ 400 copies/mL and HIV-l RNA $\leq$ 50 copies/mL) during the study period.

- Mean change from baseline and time-weighted average change (DAVG) from baseline in CD4 count.

Plasma HIV-1 RNA is widely accepted as a highly predictive surrogate marker of HIV-1 disease progression in HIV-1 infected patients.[71] The DAVG endpoint allows measurements at varying timepoints to contribute to the overall measurement of efficacy and is less sensitive to missing data than an endpoint based on a single data point. The assessment of the CD4 subset of T-cell immunophenotypes provides a well-validated marker of immune competence in HIV disease. The utility of both the virologic and immunologic markers have been validated in numerous natural history and clinical studies of antiretroviral agents in HIV-1 infected patients.

4.1.4.4.   Patient Disposition

Of 189 HIV-1 infected patients enrolled in study 902, 3 patients did not receive study medication (one in the tenofovir DF 75 mg group and two in the tenofovir DF 300 mg group). Across the four treatment groups, 26 patients (14%) discontinued study medication before week 24. For the tenofovir DF treatment groups, the discontinuation rates ranged from 9% to 16% compared with 25% for the placebo group (Table 4-2).

Of the 28 patients who were originally randomized to placebo, 21 crossed over to active treatment with tenofovir DF 300 mg once daily upon completion of week 24. The remaining seven placebo patients (25%) had discontinued by week 24. After week 24, a further 22 patients (11%) discontinued from the study prior to the week 48 visit (9 in the tenofovir DF 75 mg group, 4 in the tenofovir DF 150 mg group, 7 in the tenofovir DF

300 mg group, and 2 in the placebo/tenofovir 300 mg crossover group). Further information regarding the reasons for study discontinuation is detailed in section 4.3.2.

Table 4-2.   **Disposition of Patients Up to 24 Weeks: Study 902**

			Tenofovir DF		
	Total	**Placebo**	**75 mg**	**150 mg**	**300 mg**
Disposition	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Number of Patients Randomized	189 (100%)	28 (100%)	54 (100%)	51 (100%)	56 (100%)
Received No Study Medication	3 (2%)	0	1 (2%)	0	2 (4%)
Received ≥ 1 Dose of Study Medication & Discontinued Before Week 24	26 (14%)	7 (25%)	5 (9%)	8 (16%)	6 (11%)

4.1.4.5.   Demographic and Other Baseline Characteristics

The demographic characteristics of patients who participated in study 902 were representative of the HIV-1 infected patient population (Table 4-3). The majority of patients were male and Caucasian and the mean age was 42 years; treatment groups were well matched with respect to these characteristics.

Table 4-3.   **Demographic and Baseline Disease Characteristics (Study 902, ITT Population)**

Characteristic	Study 902 (N = 186)
Age (years)	
Mean	41.9
Median	41.1
Range	27.3 to 62.3
Gender	
Male, n (%)	171 (92%)
Female, n (%)	15 (8%)
Race	
Caucasian, n (%)	138 (74%)
Black, n (%)	24 (13%)
Hispanic, n (%)	21 (11%)
Other, n (%)	3 (2%)
CD4 count (cells/mm^3)	
Mean (SD)	374 (235)
Median	331
Range	9 to 1240
HIV-1 RNA	
Mean (copies/mL)	16,583
Mean (SD) ($\log_{10}$ copies/mL)	3.66 (0.68)
Range ($\log_{10}$ copies/mL)	1.72 to 5.76
Prior ART experience	
Mean duration (years)	4.6
HIV-1 Resistance Mutations*	
Substudy, n	184
NRTI, n (%)	173 (94%)
PI, n (%)	105 (57%)
NNRTI, n (%)	58 (32%)

*   NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor.

The mean viral load of the study populations was 3.7 $\log_{10}$ copies/mL plasma HIV-1 RNA and was consistent across the treatment groups. There was some variation across the treatment groups ranging from 298 cells/mm^3 for the placebo group to 410 cells/mm^3 for the tenofovir DF 150 mg group.

All patients had substantial prior exposure to antiretroviral therapy and most patients had evidence of resistance mutations. Baseline genotypic analysis revealed that 94% of patients

in both studies had plasma HIV-1 expressing one or more primary nucleoside-associated resistance mutations in RT (Resistance Collaborative Group definition).[72] HIV-l expressing primary protease inhibitor-associated resistance mutations were also frequent (57%) as were primary NNRTI-associated resistance mutations (32%). The incidence of mutations was similar across the treatment groups. Baseline antiretroviral therapy regimens ranged from nucleoside monotherapy to dual NRTI therapy and HAART regimens.

4.1.4.6. Efficacy Results

4.1.4.6.1. *Time Weighted Average Changes from Baseline (DAVG) in Log_{10} Copies/mL HIV-1 RNA Levels*

Statistically significant changes from baseline in plasma HIV-1 RNA levels (log_{10} copies/mL) were demonstrated for each of the tenofovir treatment groups when compared with placebo at week 4 and week 24 (Figure 4-3 and Table 4-4). The greatest effect was seen with the tenofovir DF 300 mg group with mean changes of - 0.62 log_{10} copies/mL at week 4 and - 0.58 log_{10} copies/mL at week 24 compared with a + 0.2 log_{10} copies/mL changes in the placebo group at both timepoints.

Figure 4-3.   Mean Change from Baseline in HIV-1 RNA (Study 902)
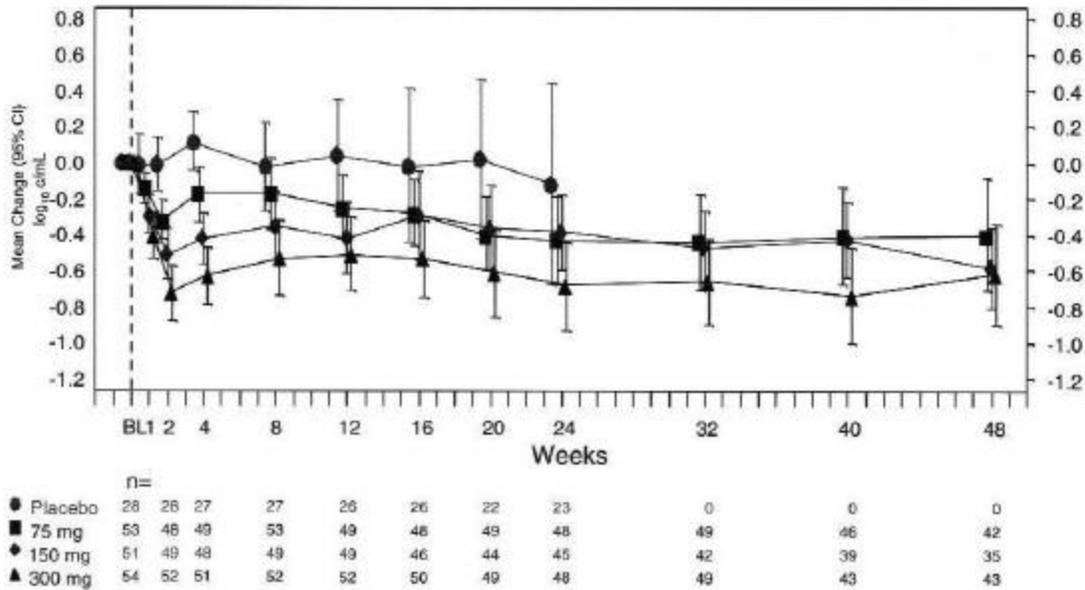
Table 4-4. **Time-Weighted Average Changes from Baseline (DAVG) in Log_{10} Copies/mL Plasma HIV-1 RNA Levels Through Weeks 4, 24 and 48: Study 902 (ITT Population)**

$DAVG_{xx}$/Group	Mean (SD)	Median	Q1, Q3[a]	P-Value[b]
$DAVG_4$				
Placebo	+0.02 (0.39)	-0.04	-0.17, +0.20	-
75 mg	-0.22 (0.35)	-0.14	-0.46, -0.03	0.008
150 mg	-0.44 (0.42)	-0.36	-0.72, -0.19	< 0.001
300 mg	-0.62 (0.49)	-0.56	-1.02, -0.25	< 0.001
$DAVG_{24}$				
Placebo	+0.02 (0.69)	+0.04	-0.20, +0.42	-
75 mg	-0.26 (0.51)	-0.16	-0.43, +0.06	0.013
150 mg	-0.34 (0.59)	-0.23	-0.74, -0.06	0.002
300 mg	-0.58 (0.63)	-0.54	-0.96, -0.12	< 0.001
$DAVG_{48}$				
75 mg	-0.33 (0.59)	-0.29	-0.59, +0.06	-
150 mg	-0.34 (0.59)	-0.29	-0.77, -0.00	-
300 mg	-0.62 (0.63)	-0.61	-1.04, -0.25	-

Note: Placebo comparison not possible at week 48 due to crossover of placebo patients to tenofovir DF 300 mg.
[a] Quartile 1 (25%), Quartile 3 (75%).
[b] p-value versus placebo, Wilcoxon rank sum test, not stratified.

Furthermore, while comparison with the placebo group at the 48-week timepoint is not possible due to the crossover to tenofovir DF 300 mg at week 24, it appears that the antiviral response to tenofovir DF in the three active treatment groups was sustained through 48 weeks (Table 4-4). Again, the greatest effect was seen with the tenofovir DF 300 mg group with a mean change of -0.62 log_{10} copies/mL.

Examination of the mean log_{10} copies/mL changes in plasma HIV-1 RNA at all time points (i.e. weeks 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, and 48), demonstrated that the tenofovir DF 150 mg and 300 mg groups, had diverged from the placebo group after one week of active treatment. At week 4, all three active treatment groups were significantly different from the placebo group (p = 0.008 for 75 mg; p < 0.001 for 150 and 300 mg). At week 24, the mean change for the tenofovir DF 300 mg group was -0.58 log_{10} copies/mL compared with a +0.02 log_{10} copies/mL mean change in the placebo group. At week 48, the mean reduction was -0.62 log_{10} copies/mL for the tenofovir DF 300 mg group.

4.1.4.6.2.   *Percentage of Patients With Plasma HIV-1 RNA at or Below Lower Limit of Quantification*

Two plasma HIV-l RNA assays were used in the study, the Amplicor HIV-1 Monitor[TM] Test with a lower limit of quantification of 400 copies/mL and the Ultrasensitive HIV-1 Monitor[TM] Test with a lower limit of quantification of 50 copies/mL. Measurements of plasma HIV-1 RNA denoted as < 50 or < 400 copies/mL were considered to have achieved values below the lower limit of quantification of the respective assay. Although the study was not powered to detect significant differences in the proportion of patients with HIV-l RNA at or below limits of quantification, these data provide further evidence of the anti-HIV-1 activity of tenofovir DF.

For the ITT population, patients without plasma HIV-1 RNA values were included as treatment failures (plasma HIV-1 RNA levels above 400 or 50 copies/mL). Using this analysis strategy, the percentages at week 24 were not significantly different between the tenofovir groups and the placebo group for both plasma HIV-1 RNA ≤ 400 copies/mL and for plasma HIV-l RNA ≤ 50 copies/mL. The proportion of patients with plasma HIV-1 RNA ≤ 400 copies/mL was 26% in the tenofovir DF 300 mg group and 21% in the placebo group; for the proportion of patients with plasma HIV-1 RNA ≤ 50 copies/mL, 13% in the tenofovir DF 300 mg group and 11% in the placebo group achieved this endpoint (Table 4-5).

An additional analysis was performed on the as-treated (AT) population which differs from the ITT population by excluding all data after permanent discontinuation of assigned study medication or addition of other antiretroviral medication. The AT analysis would, therefore, exclude data collected from patients who changed background therapy to achieve maximal suppression of viral load with the data exclusion beginning after the timepoint that patients changed background therapy.

When the AT population was analyzed for the proportion of patients with plasma HIV-1 RNA ≤ 400 copies/mL at week 24, 4% of patients in the placebo group achieved this value, compared to 19% of patients in the tenofovir DF 300 mg group. Furthermore, when the AT population was analyzed for the proportion of patients with plasma HIV-1 RNA ≤ 50 copies/mL at week 24, 11% in the tenofovir DF 300 mg group achieved this value, whereas no patients in the placebo group achieved this endpoint (Table 4-5). Although the study was not powered to detect significant differences in the proportion of patients with HIV-1 RNA ≤ 400 or ≤ 50 copies/mL, these data further indicate the anti-HIV-1 activity of tenofovir DF.

Table 4-5.   **Proportion of Patients at Week 24 With Plasma HIV-1 RNA £ 400 copies/mL and £ 50 copies/mL: Study 902 (ITT and AT Populations)**

Parameter	Placebo	Tenofovir DF		
		75 mg	150 mg	300 mg
	N (%)	N (%)	N (%)	N (%)
£ 400 Copies				
ITT	6/28 (21%)	12/53 (23%)	14/51 (27%)	14/54 (26%)
AT	1/28 (4%)	5/53 (9%)	6/51 (12%)	10/54 (19%)
£ 50 Copies				
ITT	3/28 (11%)	7/53 (13%)	6/51 (12%)	7/54 (13%)
AT	0/28 (0%)	2/53 (4%)	1/51 (2%)	6/54 (11%)

4.1.4.6.3.   *Effect on CD4 Cell Counts*

Changes in CD4 counts did not differ significantly between the active groups and the placebo group at any assessment time point during treatment. Mean changes in CD4 cell counts at the 24 week timepoint were difficult to interpret (+20 cells/mm^3, +18 cells/mm^3, 0 cells/mm^3, and -14 cells/mm^3 in the placebo, 75 mg, 150 mg, and 300 mg groups, respectively). However, the mean changes in CD4 percentage were identical and positive in the three tenofovir DF groups (+0.4%); in contrast, the placebo group exhibited a decrease from baseline of -1.1% at this time point.

All three active treatment groups had positive changes in CD4 cell count at week 48. Mean changes were +10 cells/mm^3, +20 cells/mm^3 and +11 cells/mm^3 in the 75 mg, 150 mg, and 300 mg groups, respectively. The mean changes in CD4% in the tenofovir DF groups remained positive at week 48 and had further increased in the two higher-dose groups (+0.3%, +1.2%, and +0.8% in the 75 mg, 150 mg, and 300 mg dose groups, respectively).

4.1.4.7.   Efficacy in Long-Term Use (> 48 Weeks)

In study 902 patients were given the option to continue into an open-label protocol and to receive tenofovir DF 300 mg once daily until either the drug is licensed or the Sponsor discontinues the study. At the time of the NDA submission, interim long-term data were available for the 902 extension phase. The primary objective of the 902 extension phase was to define the long-term safety profile of tenofovir DF when dosed at 300 mg once daily. In addition, plasma HIV-1 RNA and CD4 cells counts were monitored to measure virologic efficacy in extended treatment and are briefly reviewed in this section. The total number of patients who continued from the double-blind phase of study 902 into the open-label phase was 135.

Results for the extension phase demonstrate durable antiviral activity with mean decreases from baseline in plasma HIV-1 RNA observed at all visits and in all treatment groups. For the treatment groups that had received tenofovir DF throughout the double-blind phase, mean decreases from baseline in plasma HIV-1 RNA levels ranged from $\geq 0.6 - 1.2 \log_{10}$ copies/mL (Table 4-6) during the extension phase.

Table 4-6. **Changes from Baseline in Plasma HIV-1 RNA ($\log_{10}$ Copies/mL) at Weeks 72 and 96 (ITT Population): Study 902 Extension Phase**

Parameter	Dose group (0-48 Weeks/Extension)			(24-48 Weeks /Extension)
	75/300 (N = 37)	150/300 (N = 37)	300/300 (N = 42)	Placebo Crossover/300 (N = 19)
Week 72				
N	35	35	37	17
Mean (±SD)	-0.66 (1.28)	-0.64 (0.75)	-0.63 (0.98)	-0.25 (1.11)
Median	-0.69	0.66	-0.56	-0.44
Week 96				
N	18	23	18	10
Mean (±SD)	-1.17 (1.27)	-0.66 (1.24)	-0.98 (0.90)	-0.52 (1.11)
Median	-1.48	-0.68	-0.87	-0.76

Note: baseline plasma HIV-1 RNA levels for the 75/300 mg, 150/300 mg and 300/300 mg groups is defined as the average of the pre-treatment values taken after screening. Baseline for the placebo crossover/300 mg group is defined as the last assessment occurring on or before active study medication was dispensed.

All tenofovir DF treatment groups demonstrated increases from baseline in mean CD4 cell counts (Table 4-7). At week 96, the mean increase in CD4 cell count ranged from 31.4 cells/mm^3 (300/300 mg group) to 80.9 cells/mm^3 (placebo crossover/300 mg group).

Table 4-7. **Mean Change from Baseline in CD4 Cell Count at Weeks 72 and 96 (ITT Population): Study 902 Extension Phase**

	Dose Group (0-48 Weeks/Extension) Tenofovir DF			(24-48 Weeks /Extension)
	75/300 (N = 37)	150/300 (N = 37)	300/300 (N = 42)	Placebo Crossover/300 (N = 19)
Week 72				
N	35	34	37	17
Mean (±SD)	36.2±118.1	17.9±175.5	10.4±121.2	46.8±170.0
Week 96				
N	19	23	19	10
Mean (±SD)	67.1±125.4	57.7±206.0	31.4±139.7	80.9±181.2

Note: baseline plasma HIV-1 RNA levels for the 75/300 mg, 150/300 mg and 300/300 mg
groups is defined as the average of the pre-treatment values taken after screening. Baseline
for the placebo crossover/300 mg group is defined as the last assessment occurring on or
before active study medication was dispensed.

Tenofovir DF 300 mg once daily demonstrated improved virologic suppression and immunologic benefit during long-term treatment (up to 96 weeks) when used in combination antiretroviral therapy in treatment-experienced HIV-infected patients.

4.1.5. Study 907

4.1.5.1. Objectives and Study Design

The primary objective of study 907 was to evaluate the safety and efficacy of tenofovir DF 300 mg in a large population. An intensification design was again selected to enable characterization of the antiviral activity of tenofovir DF.

In this study, 552 patients were randomized in a 2:1 ratio to add tenofovir DF 300 mg once daily or placebo to their existing regimen in a blinded manner. Dose selection in this study was based on the greater antiviral effect of 300 mg tenofovir DF observed in study 901 and the results of study 902, which demonstrated a lack of dose-related toxicity.

Patients and physicians were discouraged from altering background antiretroviral regimens until at least 24 weeks post-randomization. Thereafter, changes in background antiretroviral therapy were permitted while all patients continued open-label tenofovir DF 300 mg. On completion of 48 weeks of study, patients were offered continued open-label tenofovir DF with follow-up in study 910.

Figure 4-4.　　　　**Treatment Schedule in Study 907**
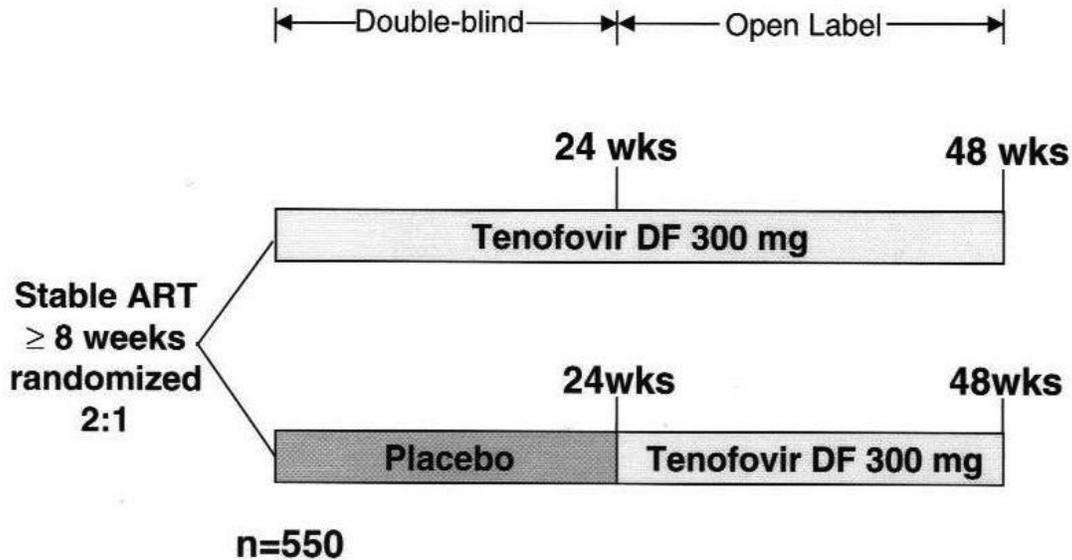
4.1.5.2.　　　Inclusion and Exclusion Criteria

As in study 902, the inclusion and exclusion criteria for study 907 were designed to select a study population that was representative of treatment experienced HIV-infected patients with a detectable viral load. The principal selection criteria was based on plasma HIV-1 RNA levels ≥ 400 copies/mL to $\leq 10,000$ copies/mL (using the Roche Standard Amplicor™ HIV-1 Monitor Test) in patients currently receiving stable antiretroviral therapy. Other key inclusion criteria were related to the requirements for adequate renal, hepatic and hematologic function. Patients were stratified by site according to HIV-1 RNA level (≤ 5000 copies/mL, $> 5,000$ copies/mL), CD4 count (≤ 200 cells/mL, > 200 cells/mL), and number of antiretroviral drugs prior to study entry (≤ 4, > 4).

4.1.5.3.　　　Efficacy Endpoints

The primary and key secondary efficacy endpoints based on these outcome are summarized below.

Primary Efficacy Endpoint

- The time-weighted average change from baseline in $\log_{10}$ copies/mL plasma HIV-1 RNA levels up to week 24 ($DAVG_{24}$).

Key Secondary Efficacy Endpoints

- The proportion of patients with plasma HIV-1 RNA levels at or below quantification limits (≤ 400 copies/mL and HIV-l RNA ≤ 50 copies/mL) during the study period.

- Mean change from baseline and time-weighted average change (DAVG) from baseline in CD4 count.

4.1.5.4. Patient Disposition

Of the 552 HIV-1 infected patients randomized in study 907, two patients discontinued the study prior to receiving any study medication and were excluded from the analysis. A total of 34 patients (6% of patients in both the tenofovir DF 300 mg and placebo treatment groups) discontinued study medication before week 24 (Table 4-8). Further information regarding reasons for study discontinuation is detailed in section 4.3.2.

Table 4-8.   **Disposition of Patients Up to 24 Weeks: Study 907**

	Total	**Placebo**	**Tenofovir DF 300 mg**
Disposition	**N (%)**	**N (%)**	**N (%)**
Number of Patients Randomized	552 (100%)	184 (100%)	368 (100%)
Received No Study Medication	2 (<1%)	2 (1%)	0
Received ≥ 1 Dose of Study Medication & Discontinued Before Week 24	34 (6%)	11 (6%)	23 (6%)

4.1.5.5. Demographics and Other Baseline Characteristics

The demographic characteristics of patients who participated in study 907 were representative of the HIV-1 infected patient population (Table 4-9). The majority of patients were male and Caucasian and the mean age was 42 years; treatment groups were well matched with respect to these characteristics.

Table 4-9.   **Demographic and Baseline Disease Characteristics (Study 907, ITT Population)**

Characteristic	Study 907 (N = 550)
Age (years)	
Mean	41.6
Median	40.0
Range	22 to 70
Gender	
Male, n (%)	469 (85%)
Female, n (%)	81 (15%)
Race	
Caucasian, n (%)	379 (69%)
Black, n (%)	92 (17%)
Hispanic, n (%)	68 (12%)
Other, n (%)	11 (2%)
CD4 count (cells/mm^3)	
Mean (SD)	427 (214)
Median	386
Range	23 to 1385
HIV-1 RNA	
Mean (copies/mL)	4440
Mean (SD) ($\log_{10}$ copies/mL)	3.36 (0.51)
Range($\log_{10}$ copies/mL)	1.70 to 4.88
Prior ART experience	
Mean duration (years)	5.4
HIV-1 Resistance Mutations*	
Substudy, n	253
NRTI, n (%)	238 (94%)
PI, n (%)	148 (58%)
NNRTI, n (%)	121 (48%)

*   NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor.

The mean viral load of the study populations 3.4 $\log_{10}$ copies/mL plasma HIV-1 RNA and was consistent across the treatment groups; the two treatment groups were similar with respect to the mean CD4 count at baseline.

All patients had substantial prior exposure to antiretroviral therapy and most patients had evidence of resistance mutations. Baseline genotypic analysis revealed that 94% of patients had plasma HIV-1 expressing one or more primary nucleoside-associated resistance

mutations in RT (Resistance Collaborative Group definition).[72] HIV-l expressing primary
protease inhibitor-associated resistance mutations were also frequent (58%) as were primary
NNRTI-associated resistance mutations (48%). The incidence of mutations was similar
across the treatment groups. Baseline antiretroviral therapy regimens ranged from nucleoside
monotherapy to dual NRTI therapy and HAART regimens.

4.1.5.6.   Efficacy Results

*4.1.5.6.1.   Time Weighted Average Changes from Baseline (DAVG) in Log_{10} copies/mL
HIV-1 RNA Levels*

The results of the primary efficacy endpoint demonstrate the significant antiviral activity of
tenofovir DF. The time-weighted average change from baseline through week 24 ($DAVG_{24}$)
for log_{10} copies/mL plasma HIV-1 RNA was significantly greater for the tenofovir DF
300 mg group (-0.61 log_{10} copies/mL) compared to the placebo group (-0.03 log_{10}
copies/mL) (Figure 4-5 and Table 4-10). Two sensitivity analyses (one with the last
observation carried forward, one with the baseline value carried forward) supported the
results of the primary analysis ($p < 0.001$ for tenofovir DF vs. placebo in both analyses).

Figure 4-5. **Mean and 95% CI in Change From Baseline in Log_{10} Plasma HIV-1 RNA Levels Over Time, Intent-to-Treat Population (Study 907)**
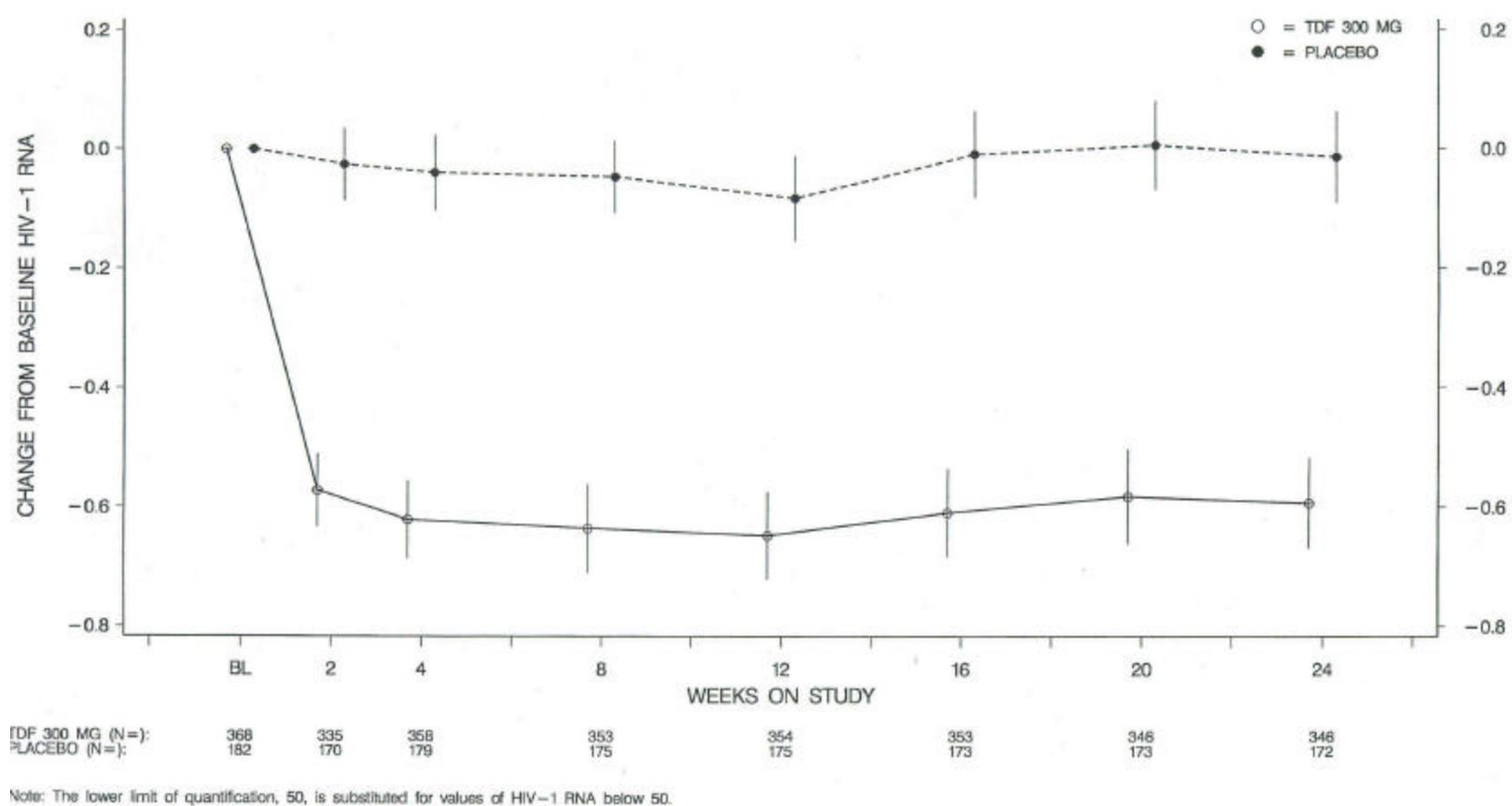
Table 4-10.   **Time-Weighted Average Changes from Baseline Through Week 24 ($DAVG_{24}$) in log_{10} Copies/mL Plasma HIV-1 RNA Levels: Study 907 (ITT Population)**

$DAVG_{24}$	Placebo	Tenofovir 300 mg
Mean (SD)	-0.03 (0.36)	-0.61 (0.61)
Median	-0.02	-0.56
Q1, Q3[a]	-0.22, +0.19	-1.07, -0.15
P-Value[b]	-	< 0.001

[a] Quartile 1 (25%), Quartile 3 (75%).

[b] p-value versus placebo, Wilcoxon rank sum test, not stratified (primary analysis); the p-value was the same using stratified Wilcoxon rank sum test

The time-weighted average changes in log_{10} copies/mL plasma HIV-1 RNA at all time points assessed (i.e. weeks 2, 4, 8, 12, 16, 20, 24), were all significantly superior for the tenofovir DF 300 mg group compared with the placebo group (p<0.001).

4.1.5.6.2.   Percentage of Patients With Plasma HIV-1 RNA at or Below Lower Limit of Quantification

Further evidence of the anti HIV-l activity of tenofovir DF is provided by the evaluation of the proportion of patients who achieved HIV-1 RNA levels at or below limits of quantification over the course of the 24-week treatment period.

In the ITT population, using the conservative strategy of patients without plasma HIV-1 RNA values as having plasma HIV-1 RNA levels above 400 or 50 copies/mL, significant treatment effects were observed with tenofovir DF treatment compared with placebo (Table 4-11). The percentage of patients with plasma HIV-l RNA ≤ 400 copies/mL at week 24 was 42% (155/368) in the tenofovir DF 300 mg group compared to 13% (23/182) in patients receiving placebo. The percentage of patients with plasma HIV-l RNA ≤ 50 copies/mL at week 24 was 21% (76/368) in the tenofovir DF 300 mg group compared to 1% (2/182) in patients receiving placebo (Table 4-11). The results of this analysis using the AT population, which included all patients who received at least one dose of study medication but excluded all data after discontinuation of assigned study medication and/or addition of other antiretroviral therapy, were similar to the results using the ITT population.

Table 4-11. **Proportion of Patients at Week 24 with Plasma HIV-1 RNA £ 400 copies/mL and £ 50 copies/mL: Study 907 (ITT Population)**

HIV-1 RNA	Placebo	Tenofovir DF 300 mg	P-Value*
£ 400 copies/mL	23/172 (13%)	155/346 (45%)	< 0.0001
£ 50 copies/mL	2/155 (1%)	7/325 (22%)	< 0.0001

* CMH General Association Test

4.1.5.6.3. *Effect on CD4 Cell Counts*

The efficacy of tenofovir DF in this population of HIV-1 infected patients was also evident from the immunologic effect on the level of CD4 cell counts. Treatment with tenofovir DF resulted in mean time-weighted average change from baseline in CD4 count of 12 cells/mm^3 occurring at week 24. The change at week 24 for CD4 count represented a significant difference between tenofovir DF 300 mg versus placebo (p-value = 0.0008). A statistically significant difference favoring tenofovir DF was seen at every on-treatment assessment time point for the time-weighted average change from baseline in CD4 cell count (Table 4-12).

The mean and 95% CI in change from baseline in CD4 cell counts (cells/mm^3) over time is presented in Figure 4-6.

Table 4-12. **Time-Weighted Average Change from Baseline in CD4 Cell Counts: Study 907 (ITT Population)**

Visit	Placebo (N = 182)	Tenofovir DF (N = 368)	P-Value*
Baseline Cell Count	447.1 (216.8)	417.4 (211.7)	0.19
	Mean (± SD) Time-Weighted Average Change from Baseline (cells/mm^3)		
Week 4	-14.1 (90.9)	6.4 (97.9)	0.01
Week 8	-13.2 (79.5)	6.6 (84.8)	0.004
Week 12	-12.0 (82.7)	9.2 (81.5)	0.003
Week 16	-12.2 (82.8)	10.7 (78.8)	0.001
Week 20	-11.0 (89.7)	12.2 (79.0)	0.0007
Week 24	-10.6 (88.4)	12.6 (78.4)	0.0008

* Wilcoxon rank sum test

Figure 4-6.   **Mean and 95% CI in Change from Baseline in CD4 Cell Counts (Cells/mm^3) Over Time, Intent-to-Treat Population (Study 907)**

	BL	4	8	12	16	20	24
TDF 300 MG (N=):	368	352	356	352	347	346	336
PLACEBO (N=):	182	177	172	175	170	169	170
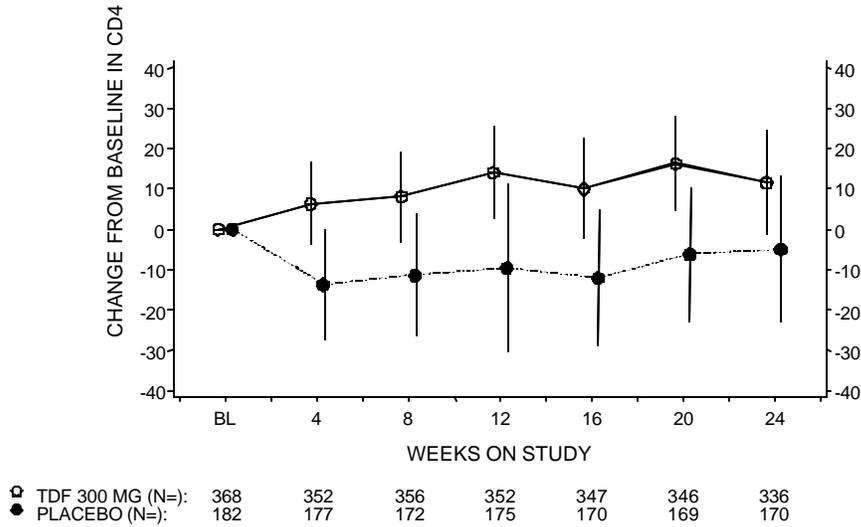
4.1.5.6.4.   *Subgroup Analyses*

The anti-HIV-1 activity of tenofovir DF in study 907 was confirmed across a range of subgroups based on demographic characteristics (age, gender and race) and baseline disease status (HIV-l RNA level, CD4 cell count). All subgroup analyses were evaluated using the time-weighted average change from baseline through week 24 (DAVG$_{24}$) in plasma HIV-1 RNA (log$_{10}$ copies/mL) and p-values were based on the Wilcoxon rank sum test.

The results of these analyses demonstrate a consistent treatment effect across all subgroups evaluated, that is, in all subgroups there was a significant difference in DAVG$_{24}$ in plasma HIV-1 RNA (log$_{10}$ copies/mL) for patients receiving tenofovir DF compared to patients receiving placebo (Table 4-13). Of the population characteristics investigated, none was predictive of a different antiviral effect than that observed for the group as a whole.

Table 4-13. **Time-Weighted Average Changes from Baseline up to Week 24 (DAVG$_{24}$) in log$_{10}$ Copies/mL Plasma HIV-1 RNA Levels by Demographic and Disease Characteristic Subgroup: Study 907 (ITT Population)**

Characteristic	Subgroup		DAVG$_{24}$		
			Placebo	**TDF 300 mg**	**P-Value**
Age	≤40 years	n Mean (SD) Median Range	93 -0.05 (±0.38) -0.02 -1.28 to1.01	182 -0.64 (±0.62) -0.63 -2.17 to 1.37	<0.0001
	>40 years	n Mean (SD) Median Range	89 -0.01 (±0.33) -0.03 -1.35 to 0.85	185 -0.59 (±0.60) -0.53 -2.09 to 1.03	<0.0001
Sex	Male	n Mean (SD) Median Range	160 -0.02 (±0.36) -0.02 -1.35 to 1.01	309 -0.61 (±0.61) -0.56 -2.17 to 1.37	<0.0001
	Female	n Mean (SD) Median Range	22 -0.08 (±0.38) -0.11 -1.28 to 0.56	59 -0.66 (±0.60) -0.59 -2.03 to 0.35	0.0002
Race	Caucasian	n Mean (SD) Median Range	118 -0.02 (±0.37) -0.02 -1.28 to 1.01	261 -0.60 (±0.60) -0.56 -2.17 to 1.37	<0.0001
	Non-Caucasian	n Mean (SD) Median Range	64 -0.05 (±0.34) -0.04 -1.35 to 0.85	106 -0.65 (±0.64) -0.58 -1.85 to 1.16	<0.0001
HIV-1 RNA	< 5000 copies/mL	n Mean (SD) Median Range	139 0.03 (±0.33) 0.01 -0.82 to 1.01	268 -0.59 (±0.61) -0.56 -1.85 to 1.37	<0.0001
	≥ 5000 copies/mL	n Mean (SD) Median Range	43 -0.22 (±0.38) -0.14 -1.35 to 0.40	99 -0.67 (±0.61) -0.57 -2.17 to 0.58	<0.0001
CD4 Count	< 200 cells/mm^3	n Mean (SD) Median Range	21 0.05 (±0.37) -0.03 -0.85 to 0.84	45 -0.39 (±0.55) -0.37 -2.03 to 0.80	0.0007
	≥ 200 cells/mm^3	n Mean (SD) Median Range	161 -0.04 (±0.35) -0.02 -1.35 to 1.01	322 -0.64 (±0.61) -0.58 -2.17 to 1.37	<0.0001

4.1.6.   Efficacy Conclusions

Two placebo-controlled clinical studies of tenofovir DF (studies 902 and 907) demonstrate that tenofovir DF 300 mg once daily, when used in combination with other antiretroviral drugs, is effective for the treatment of HIV-1 infected patients who have failed or are intolerant to nucleoside analog therapy, or are not controlled by their current antiretroviral regimen. Efficacy was demonstrated based on significant changes in established and validated surrogate markers for HIV-1 disease (plasma HIV-1 RNA) and immune competence (CD4 cell count), in patients who received tenofovir DF 300 mg daily in combination with standard antiretroviral therapy at 24 weeks (studies 902 and 907) and for up to 48 weeks (study 902 only).

The principal clinical efficacy findings in study 907, the primary phase 3 study, at week 24 are as follows:

- Treatment with tenofovir DF 300 demonstrated a significant reduction in the time-weighted average change from baseline ($DAVG_{24}$) in $\log_{10}$ copies/mL plasma HIV-1 RNA level (-0.61 $\log_{10}$ copies/mL) compared with placebo (-0.03 $\log_{10}$ copies/mL).

- A significantly higher proportion of tenofovir DF patients achieved plasma HIV-1 RNA levels at or below the limit of quantification ($\leq$ 400 copies/mL 42%; $\leq$ 50 copies/mL 21%) compared with placebo treatment ($\leq$ 400 copies/mL 13%; $\leq$ 50 copies/mL 1%).

- There was a significant increase in the time-weighted average change from baseline ($DAVG_{24}$) in the CD4 cell count.

- Consistently significant reductions from baseline in $\log_{10}$ copies/mL plasma HIV-1 RNA levels were observed in all subgroups based on demographic and disease characteristics.

- Tenofovir DF 300 mg daily demonstrated significant anti-HIV-1 activity in patients with HIV-1 expressing resistance mutations associated with nucleoside and non-nucleoside reverse transcriptase inhibitors and protease inhibitors.

The principal efficacy findings in study 902, the phase 2 dose-finding study, are as follows:

- Statistically significant reductions in the time-weighted average change from baseline in $\log_{10}$ copies/mL plasma HIV-1 RNA levels were demonstrated for each of the tenofovir DF treatment groups when compared with placebo at week 4 and week 24.

- The greatest antiviral effect was seen with tenofovir DF 300 mg with mean changes of -0.62 and -0.58 $\log_{10}$ copies/mL at week 4 and 24, respectively, providing further support for the selection of this dose for the treatment of HIV-infected patients.

- The antiviral response was sustained through 48 weeks as demonstrated by the mean change from baseline of -0.62 $\log_{10}$ copies/mL at week 48 for the tenofovir DF 300 mg treatment group.

- Significant anti-HIV-1 activity was demonstrated in patients with HIV-1 expressing resistance mutations associated with nucleoside and non-nucleoside reverse transcriptase inhibitors and protease inhibitors.

The effects of tenofovir DF 300 mg daily in study 907 with respect to plasma HIV-1 RNA and CD4 cell count when added to intensify therapies due to incomplete suppression of virus are generally consistent with results reported from a similar placebo-controlled phase 3 study of abacavir 300 mg twice daily when added to failing background therapy.[74] The abacavir study population was similar with baseline plasma HIV-1 RNA levels between 400 and 50,000 copies/mL and CD4 counts of at least 100 cells/mm^3. However, only 6% of the patients in this trial had 18 months or more of prior antiretroviral therapy, making this population notably less antiretroviral-experienced than the populations in study 907.

Median plasma HIV-1 RNA levels at baseline were 3.68 $\log_{10}$ copies/mL and 3.53 $\log_{10}$ copies/mL for the abacavir and placebo groups, respectively. In study 907, median baseline plasma HIV-1 RNA levels were 3.37 $\log_{10}$ copies/mL for both tenofovir DF-treated patients and for those receiving placebo. At week 16, the addition of abacavir to stable background therapy resulted in a median change in plasma HIV-1 RNA from baseline of -0.44 $\log_{10}$ copies/mL, and the proportion of patients with plasma HIV-1 RNA ≤ 400 copies/mL was 39%. By comparison, in study 907 at week 16, the median change in plasma HIV-1 RNA from baseline was -0.57 $\log_{10}$ copies/mL, and the proportion of patients with HIV-1 RNA ≤ 400 copies/mL was 43% in patients treated with tenofovir DF.

At week 16, abacavir patients had a change in median CD4 count from baseline of 30 cells/mm^3 (range, -285 to 485 cells/mm^3), which did not differ significantly from patients randomized to placebo (p = 0.093). In study 907, the median change from baseline in CD4 count observed at week 16 with tenofovir DF 300 mg per day was +10 cells/mm^3 (range, -411 to 725). The results of both studies suggest that in treatment-experienced patients, the addition of a single antiretroviral agent to stable background therapy can result in significant reductions in plasma HIV-1 RNA with only small changes occurring in CD4 counts.

In conclusion, tenofovir DF 300 mg once daily demonstrates statistically significant antiviral activity in highly treatment-experienced HIV-infected patients with corresponding baseline genotypic evidence of nucleoside-resistant virus.

4.2.   Virology

Results of in vitro resistance studies are summarized as an introduction to this section. HIV virology studies have been performed in conjunction with clinical trials of tenofovir and tenofovir DF for the treatment of HIV-infected patients. The objectives of these virology studies were to:

- Determine whether RT resistance mutations develop during tenofovir DF therapy.

- Determine whether any RT mutations that develop correlate with reduced susceptibility of the mutant HIV to tenofovir in vitro or with increasing HIV viral load in vivo.

- Determine whether the baseline HIV RT genotype in antiretroviral experienced patients affects treatment response to tenofovir DF.

Results from clinical resistance analyses demonstrate the significant antiviral efficacy of tenofovir through 48 weeks in patients with extensive nucleoside resistance in their HIV at baseline, and a low incidence of genotypic or phenotypic resistance to tenofovir arising during 24-48 weeks of tenofovir DF therapy

4.2.1. Summary of Nonclinical Resistance

Results of in vitro resistance evaluations show:

- Tenofovir remains active (within 2-fold of wild-type) against recombinant mutant molecular clones of HIV-1 expressing nucleoside-resistance mutations including: didanosine resistance (L74V), zalcitabine resistance (T69D), zidovudine resistance (D67N + K70R, D67N + K70R + K219Q, or T215Y) or multinucleoside drug resistance (Q151M complex) mutations in HIV-1 RT.[8, 75-77]

- Tenofovir shows slightly increased activity against HIV-1 expressing the abacavir/lamivudine resistance mutation M184V or the combination of the high-level zidovudine resistance mutation T215Y and M184V.[6, 8]

- Tenofovir showed activity against all common forms of non-nucleoside-resistant, and protease inhibitor-resistant HIV.[8, 75-77]

- Tenofovir showed a 3 to 4-fold decreased activity against HIV-1 expressing the K65R mutation. K65R was selected by tenofovir in vitro and infrequently by other antiretroviral drugs in vivo.[8]

- HIV expressing the multinucleoside-resistant T69S double amino acid insertion mutations (T69S Ins) were resistant to tenofovir.[78, 79] Intermediate susceptibility to tenofovir was observed when these insertions were combined with M184V.

- The removal of nucleoside chain-terminator inhibitors by HIV RT using a pyrophosphate acceptor molecule or a similar mechanism using ATP as an acceptor have been proposed as mechanisms of nucleoside RT resistance.[80, 81] Tenofovir was inefficiently removed by the ATP-dependent unblocking mechanism by both wild-type RT and an RT mutant with the ZDV resistance mutations D67N + K70R + T215Y that demonstrated increased removal of zidovudine and stavudine.[82]

4.2.2.   Study 902 Virology Substudy

A virology substudy of study 902 included all patients who enrolled in the trial (n = 189); final analyses consisted of the ITT population (n = 186). Both the HIV-1 RT and protease genes from banked plasma samples from all patients were genotypically analyzed at baseline, week 24, week 48, or early termination. Phenotypic analyses of tenofovir susceptibility were performed at baseline and week 48 or early termination for all patients who were assigned to tenofovir DF 300 mg. Additional phenotypic analyses were performed for patients who developed nucleoside-associated RT mutations, including all patients who developed the K65R mutation in RT.

Baseline HIV genotypic data were obtained from 184 of 186 patients in the ITT population; plasma HIV from two patients, both in the placebo arm, failed to generate a sufficient PCR product for genotypic analysis. As noted in Table 4-3, 94% of analyzed patients had plasma HIV expressing one or more primary nucleoside-associated resistance mutations in RT (Resistance Collaborative Group definition),[72] 57% expressed primary PI-associated resistance mutations, and 32% expressed primary NNRTI-associated resistance mutations. Most patients (74%) had HIV with typical ZDV/thymidine analog-associated resistance mutations at RT codons 41, 67, 70, 210, 215 or 219 (mean of 2.8 mutations); 66% had HIV with the lamivudine/abacavir-associated M184V/I mutations; and 47% had both of these types of resistance mutations.

4.2.2.1.   HIV RNA Response to Tenofovir DF Therapy by Baseline HIV Genotype

The HIV RNA responses among patients with HIV expressing specific types of resistance mutations at baseline are shown in Table 4-14 in an intent-to-treat analysis (excluding two patients without baseline genotypic data). The decrease in plasma HIV RNA among patients taking tenofovir DF 300 mg was similar among patients expressing or not expressing ZDV- or lamivudine-associated (M184V) resistance mutations in their HIV. Patients with HIV expressing the M184V mutation in the absence of ZDV-associated mutations had the largest decline in HIV RNA among all genotypic groups (-0.91 $\log_{10}$ copies/mL $DAVG_{24}$ for tenofovir DF 300 mg). These responses were durable through 48 weeks of therapy. Patients with HIV expressing the high-level ZDV resistance mutation T215Y or F (51% of patients), NNRTI-associated, or PI-associated resistance mutations also responded durably to tenofovir DF 300 mg. Results of an As-Treated analysis were similar.

Table 4-14. **HIV RNA Responses by Baseline Resistance Mutations in Study 902 (N = 184, Virology Intent-to-Treat[1])**

| Baseline Mutation Group | Mean DAVG$_{24}$[2] (n) | | | | p-Value[3] | Mean DAVG$_{48}$[2,4] (n) |
	Placebo	TDF 75 mg	TDF 150 mg	TDF 300 mg		TDF 300 mg
All Patients	+0.02 (28)[5]	-0.26 (53)	-0.34 (51)	-0.58 (54)	< 0.001	-0.62 (54)
No M184V	+0.28 (10)	-0.32 (15)	-0.30 (15)	-0.48 (22)	0.001	-0.57 (22)
M184V	-0.20 (16)	-0.23 (38)	-0.36 (36)	-0.65 (32)	0.025	-0.64 (32)
M184V / No ZDV-R[6]	+0.07 (4)	-0.31 (13)	-0.55 (9)	-0.91 (9)	0.025	-0.82 (9)
No ZDV-R[6]	+0.19 (6)	-0.30 (16)	-0.64 (12)	-0.61 (14)	0.019	-0.55 (14)
ZDV-R[6]	-0.08 (20)	-0.24 (37)	-0.25 (39)	-0.57 (40)	0.003	-0.64 (40)
ZDV-R[6] / No M184V	+0.24 (8)	-0.32 (12)	-0.14 (12)	-0.60 (17)	0.002	-0.72 (17)
ZDV-R[6] + M184V	-0.29 (12)	-0.20 (25)	-0.30 (27)	-0.55 (23)	0.217	-0.58 (23)
T215Y/F	-0.03 (14)	-0.16 (25)	-0.28 (29)	-0.47 (26)	0.046	-0.61 (26)
T215Y/F / No M184V	+0.29 (6)	-0.21 (9)	-0.26 (9)	-0.54 (11)	0.018	-0.72 (11)
T215Y/F + M184V	-0.27 (8)	-0.13 (16)	-0.28 (20)	-0.41 (15)	0.723	-0.53 (15)
T69D/N	-0.53 (5)	-0.15 (9)	-0.40 (6)	-0.74 (5)	0.676	-0.80 (5)
L74V/I	+0.09 (7)	-0.36 (6)	-0.35 (4)	+0.09 (3)	1.000	-0.11 (3)
NNRTI-R[7]	+0.23 (8)	-0.24 (14)	-0.24 (19)	-0.52 (17)	0.004	-0.63 (17)
Protease Inhibitor-R[8]	-0.03 (18)	-0.29 (27)	-0.29 (27)	-0.61 (33)	0.001	-0.66 (33)

1 Excluding 2 patients without baseline genotypic data.
2 Average HIV RNA change from baseline through week 24 (DAVG$_{24}$) or week 48 (DAVG$_{48}$) in log$_{10}$ copies/mL.
3 Wilcoxon rank sum test comparing TDF 300 mg to placebo in the same mutation group.
4 During weeks 24 through 48, placebo patients received TDF 300 mg, precluding placebo comparisons.
5 Includes two patients without baseline genotypic data.
6 Zidovudine resistance mutations are M41L, D67N, K70R, L210W, T215Y/F or K219Q in RT.
7 K103N or Y181C in RT.
8 Any amino acid substitution at codons 30, 48, 50, 82, 84, or 90 in protease.
Data from reference: [83]

Fewer patients had HIV expressing mutations at other nucleoside-associated RT resistance residues, which precluded definitive analyses of the effects of these mutations on response to therapy with tenofovir DF. There was only a single patient whose HIV expressed the K65R

RT mutation at baseline in this study. Thus, in this study, it is not possible to determine the effect of a pre-existing K65R mutation on response to tenofovir DF therapy.

4.2.2.2. Development of RT Mutations

Post-baseline genotypic data (week 24, week 48, or early termination) were obtained from 159 of 186 patients who received study medication (110 patients at week 48); the remaining patients had insufficient HIV RNA to genotype (n = 27).

Development of Nucleoside-Associated RT Mutations by Treatment Arm

Any patient with a post-baseline plasma sample showing a mutation resulting in any amino acid substitution at any of the 16 amino acid residues in RT associated with nucleoside resistance (Resistance Collaborative Group definition; residues 41, 62, 65, 67, 69, 70, 74, 75, 77, 115, 116, 151, 184, 210, 215 and 219 of RT) was considered to have developed a nucleoside-associated RT mutation. Of the 159 genotypically evaluable patients, 79 patients developed one or more of the mutations during the 48-week study.

The distribution of patients who developed nucleoside-associated RT mutations across the placebo and three treatment arms of the study is shown in Table 4-15. During the placebo-controlled phase, 14% of patients in the placebo arm developed a nucleoside-associated RT mutation versus 21%, 37%, and 22% of patients in the tenofovir DF 75 mg, 150 mg, and 300 mg treatment arms, respectively. From logistic regression analyses using the Wald chi-squared test and from Fisher's exact test comparisons, there were no statistically significant differences in the development of nucleoside-associated RT mutations between placebo and each of the treatment arms. Similarly, in patients developing RT mutations through 48 weeks, there was no dose-response across the treatment arms with 34%, 57%, and 39% of patients developing a new nucleoside-associated RT mutation in the three dose groups originally randomized to tenofovir DF therapy. These comparisons suggest that background antiretroviral therapy and not tenofovir DF was responsible for the development of these mutations. Moreover, patients in this study who developed nucleoside-associated RT mutations in the tenofovir DF 300 mg treatment group showed continued viral load suppression in HIV RNA at both week 24 and week 48 ($DAVG_{24}$ = -0.59 $\log_{10}$ copies/mL and $DAVG_{48}$ = -0.59 $\log_{10}$ copies/mL, n = 21) similar to the -0.62 $\log_{10}$ copies/mL decrease in $DAVG_{48}$ observed for all patients treated with tenofovir DF 300 mg (n = 54) or the -0.63 $\log_{10}$ copies/mL $DAVG_{48}$ decrease observed for patients not developing a nucleoside-associated RT mutation (n = 33).

Table 4-15.   **Development of Nucleoside-Associated RT Mutations by Treatment Arm in Study 902**

	Treatment Arm				
	Placebo	**TDF 75 mg**	**TDF 150 mg**	**TDF 300 mg**	**p-Value[1]**
% of Patients Developing RT Mutations by Week 24 (Number of Patients)	14% (4/28)	21% (11/53)	37% (19/51)	22% (12/54)	0.34
% of Patients Developing RT Mutations by Week 48 (Number of Patients)	NA	34% (18/53)	57% (29/51)	39% (21/54)	0.62

1 Logistic regression analysis and Wald chi-squared test comparing placebo to tenofovir DF (week 24) or across tenofovir DF doses (week 48).
2 At week 24, patients in the placebo arm began receiving TDF 300 mg treatment.
Data from reference: [83]

Development of ZDV/Thymidine Analog-Associated RT Mutations

The specific amino acid substitutions observed among the patients who developed nucleoside-associated RT mutations also suggest that background therapy was responsible for the development of these mutations (Table 4-16). The majority of the patients (63 of 79) developed typical ZDV-associated mutations while taking either zidovudine, stavudine, abacavir, or lamivudine concomitantly, and the majority of these patients (48 of 63) were adding additional ZDV-associated mutations onto a background of pre-existing ZDV resistance mutations. The capacity of stavudine and abacavir to also select for "zidovudine-associated" mutations in vivo has been established.[84-88]

Table 4-16.   **Development of Antiretroviral-Associated HIV Mutations by Week 48 (Intent-to-Treat, N = 186) in Study 902**

RT and Protease Resistance Mutations Developing	Percent of Patients (n)					
	Placebo[1] (N = 28)					
	Up to Week 24	Week 24 to 48	TDF 75 mg (N = 53)	TDF 150 mg (N = 51)	TDF 300 mg (N = 54)	Total (N = 186)
Nucleoside-Associated: (concomitant ART)	14% (4)	25% (7)	34% (18)	57% (29)	39% (21)	42% (79)
M41L, D67N/G, K70R, L210W/S, T215Y/F/I or K219E/Q/N (d4T, ZDV, ABC or 3TC)	11% (3)	21% (6)	25% (13)	45% (23)	33% (18)	34% (63)[2]
M184V (3TC)		4% (1)	2% (1)	6% (3)	2% (1)	3% (6)
T69D/N (ZDV, ABC or d4T)			6% (3)	6% (3)		3% (6)
L74V/I (ddI, ABC or 3TC)			4% (2)	4% (2)	2% (1)	3% (5)
K65R (ddI or ABC)		4% (1)		2% (1)	4% (2)	2% (4)
A62V (ZDV or d4T)		4% (1)	4% (2)		2% (1)	2% (4)
V75L/A (ddI or d4T)	4% (1)		2% (1)		2% (1)	2% (3)
Y115F (ABC)			4% (2)			1% (2)
F77L (ZDV)					2% (1)	0.5 % (1)
Q151M (ABC and d4T)			2% (1)			0.5 % (1)
Primary NNRTI-Associated (any change at residues 103 or 181 in RT)		7% (2)	8% (4)	8% (4)	9% (5)	8% (15)[3]
Primary PI-Associated (any change at residues 30, 32, 48, 82, 84, or 90 in protease)	11% (3)	4% (1)	6% (3)	12% (6)	6% (3)	9% (16)[4]

1   Patients on placebo up to week 24, TDF 300 mg between weeks 24 and 48.
2   48 of these patients also had ZDV-associated mutations at codons 41, 67, 70, 210, 215, or 219 at baseline.
3   5 of these patients also had primary NNRTI-associated resistance mutations at baseline.
4   11 of these patients also had primary PI-associated resistance mutations at baseline.

Data from reference: [83]

Development of K65R RT Mutations

Four patients (2% of all patients) developed the K65R mutation, an RT mutation associated with zalcitabine, didanosine, and abacavir *in vivo*, and also selected by tenofovir *in vitro*.[75, 89-91] All four patients were taking either didanosine (n = 3) or abacavir (n = 1) concomitantly with tenofovir DF (150 mg or 300 mg). Phenotypic analysis of HIV from all four patients was performed and compared to the patient's baseline HIV susceptibility to tenofovir. Recombinant HIV from the analyzed patients demonstrated a 2.8 to 3.9-fold reduction in tenofovir susceptibility after the acquisition of the K65R mutation, consistent with results from site-directed recombinant viruses expressing only the K65R mutation. Finally, there was no consistent pattern of HIV RNA increases observed coincident with the development of K65R in the patients.

4.2.2.3.   Baseline Phenotypic Analyses

Baseline phenotypic analyses were attempted for all patients treated with tenofovir DF 300 mg at study entry (n = 54); successful phenotypic results were generated for 53 of these patients. Among these 53 patients with baseline phenotypic results, the mean baseline susceptibility was 1.9 fold above wild-type control for tenofovir (range 0.4 - 6.0) versus > 13.8-fold above wild-type for ZDV (range 0.3 - > 150) and > 24.1-fold above wild-type control for lamivudine (range 0.2 - > 54.5). There were a total of four patients who had HIV with > 4-fold reduced susceptibility to tenofovir, including the single patient with the K65R, whose HIV demonstrated 5.2-fold reduced susceptibility to tenofovir. No patients had HIV with > 10-fold reduced susceptibility to tenofovir at baseline.

The HIV RNA response among various strata of baseline susceptibility to tenofovir is shown in Table 4-17. Patients with baseline tenofovir susceptibility within three-fold of wild-type all responded with $\geq 0.5 \log_{10}$ decreases in HIV RNA which were durable through week 48. In the intent-to-treat analyses, patients with 3- to 4-fold reduced susceptibility to tenofovir responded to tenofovir DF 300 mg therapy (-0.55 $\log_{10}$ copies/mL DAVG$_{24}$ ITT, -0.32 $\log_{10}$ copies/mL DAVG$_{24}$ AT). There were only four patients with > 4-fold reduced susceptibility to tenofovir at baseline and, as a group, these patients did not appear to respond to tenofovir DF 300 mg therapy.

Table 4-17.   **Response to Tenofovir DF 300 mg Therapy by Baseline Tenofovir Susceptibility in Study 902**

Baseline Tenofovir Susceptibility (-fold change from wild-type)	N	$DAVG_{24}$[1]	$DAVG_{48}$[1]
≤ 1.0	14	-0.71	-0.61
> 1.0 and ≤ 2.0	21	-0.63	-0.68
> 2.0 and ≤ 3.0	8	-0.57	-0.56
> 3.0 and ≤ 4.0	6	-0.55	-0.55
> 4.0	4	-0.17	-0.72
All Patients Analyzed	53	-0.60	-0.63

1   Mean $DAVG_{xx}$ for all patients in group ($\log_{10}$ copies/mL).

Data from reference: [83]

4.2.3.   Study 907 Virology Substudy

Approximately 50% of enrolled patients were randomly selected and included in the genotypic analyses substudy (n = 274) and 50% of these patients were included in the phenotypic analyses substudy (n = 137). Both the HIV-1 RT and protease genes from banked plasma samples from the patients in the genotyping substudy were genotypically analyzed at baseline and week 24 or early termination. Phenotypic analyses of susceptibility to tenofovir and all approved nucleoside analogs were performed at baseline and week 24 or early termination for all patients in the phenotyping substudy. Baseline HIV genotypic data were obtained from 253 of the 274 patients in the virology genotyping substudy; plasma HIV from 21 patients (14 tenofovir DF; 7 placebo) failed to yield a sufficient PCR product for genotypic analysis.

As noted in Table 4-18, 94% of analyzed patients had plasma HIV expressing one or more primary nucleoside-associated resistance mutations in RT (Resistance Collaborative Group definition),[72] 58% expressed primary PI-associated resistance mutations, and 48% expressed primary NNRTI-associated resistance mutations. Most patients (69%) had HIV with typical ZDV/thymidine analog-associated resistance mutations at RT codons 41, 67, 70, 210, 215, or 219 (mean of 2.8 mutations); 68% had HIV with the lamivudine/abacavir-associated M184V/I mutations; and 45% had both of these types of resistance mutations. The prevalence of each of these baseline resistance mutations is similar across the two treatment arms in the study.

Table 4-18.   **Baseline Genotypic Analysis in Study 907 (N = 253, Virology Intent-to-Treat)**

RT and Protease Resistance Mutations at Baseline	Percent of Patients (n)		
	Placebo (N = 84)	Tenofovir DF (N = 169)	Total (N = 253)
Nucleoside-Associated[1]:	94% (79)	94% (159)	94% (238)
ZDV-R (M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N)	73% (61)	67% (114)	69% (175)
M184V/I	64% (54)	70% (118)	68% (172)
T215Y/F	46% (39)	47% (80)	47% (119)
M184V/I + ZDV-R	45% (38)	44% (75)	45% (113)
T69D/N	17% (14)	12% (20)	13% (34)
L74V/I	11% (9)	9% (15)	9% (24)
A62V	1% (1)	3% (15)	2% (6)
V75T/I	1% (1)	2% (4)	2% (5)
K65R		3% (5)	2% (5)
Q151M	2% (2)	1% (2)	2% (4)
T69S Insertions	0% (0)	1% (2)	1% (2)
Primary NNRTI-Associated[2]	52% (44)	46% (77)	48% (121)
Primary PI-Associated[3]	62% (52)	57% (96)	58% (148)

1   Mutations M41L, A62V, K65R, D67N, T69D/N, K70R, L74V/I, V75T/I, F77L, Y115F, F116Y, Q151M, M184V, L210W, T215Y/F or K219Q/E/N in RT.

2   NNRTI resistance mutations are K103N, V106A, V108I, Y181C/I, Y188C/L/H, G190A/S/E or P236L in RT.

3   Protease inhibitor resistance mutations are D30N, V32I, G48V, I50V, V82A/F/T/S, I84V or L90M in protease.

Data from reference: [92]

4.2.3.1.   HIV RNA Response to Tenofovir DF Therapy by Baseline HIV Genotype

The HIV RNA responses among patients with HIV expressing specific types of resistance mutations at baseline are shown in Table 4-19 in an intent-to-treat analysis. In both intent-to-treat and as-treated analyses,[92] treatment with tenofovir DF resulted in statistically significant decreases in plasma HIV RNA among patients expressing ZDV-associated or lamivudine (M184V)-associated resistance mutations in their HIV. There appeared to be slightly

improved responses in patients expressing the M184V mutation versus those without M184V, and slightly diminished response in patients expressing ZDV-associated mutations in their HIV versus those without ZDV-associated mutations, but the net treatment differences were not statistically significant.[92]

Table 4-19. **HIV RNA Responses by Baseline Resistance Mutations in Study 907(N = 253, Virology Intent-to-Treat)**

Baseline Mutation Group	Mean $DAVG_{24}$[1] (n)		Net Treatment Effect [2]	P-Value[3]
	Placebo	Tenofovir DF		
All Patients	-0.03 (84)	-0.59 (168)	-0.56	< 0.0001
No M184V	+0.02 (30)	-0.40 (51)	-0.42	0.0006
M184V	-0.05 (54)	-0.68 (117)	-0.63	< 0.0001
M184V / No ZDV-R[4]	-0.16 (16)	-0.97 (42)	-0.81	< 0.0001
No ZDV-R[4]	-0.18 (23)	-0.85 (54)	-0.67	< 0.0001
ZDV-R[4]	+0.03 (61)	-0.47 (114)	-0.50	< 0.0001
ZDV-R[4] / No M184V	+0.09 (23)	-0.39 (39)	-0.48	0.0002
ZDV-R[4] + M184V	-0.01 (38)	-0.51 (75)	-0.50	< 0.0001
T215Y/F	+0.05 (39)	-0.32 (80)	-0.37	< 0.0001
T215Y/F/ No M184V	+0.08 (18)	-0.31 (33)	-0.39	0.002
T215Y/F + M184V	+0.01 (21)	-0.32 (47)	-0.33	0.0018
T69D/N	+0.08 (14)	-0.42 (20)	-0.50	0.002
L74V/I	+0.13 (9)	-0.22 (15)	-0.35	0.027
K65R	0	+0.12 (5)	+0.12	NA[7]
Q151M	+0.05 (2)	+0.38 (2)	+0.33	0.698
T69S Insertions	0	+0.29 (2)	+0.29	NA[7]
NNRTI-R[5]	+0.02 (44)	-0.49 (77)	-0.51	< 0.0001
Protease Inhibitor-R[6]	-0.00 (52)	-0.55 (96)	-0.55	< 0.0001

1   Average HIV RNA change from baseline through week 24 ($DAVG_{24}$) in $\log_{10}$ copies/mL.

2   Difference between $DAVG_{24}$ values of tenofovir DF- versus placebo-treated patients.

3   Wilcoxon rank sum test comparing tenofovir DF to placebo in the same mutation group.

4   Zidovudine resistance mutations are M41L, D67N, K70R, L210W, T215Y/F or K219Q/E/N in RT.

5   NNRTI resistance mutations are K103N, V106A, V108I, Y181C/I, Y188C/L/H, G190A/S/E or P236L in RT.

6   Protease inhibitor resistance mutations are D30N, V32I, G48V, I50V, V82A/F/T/S, I84V or L90M in protease.

7   Not applicable; no comparator patients in placebo arm.

Data from reference: [92]

Patients with HIV expressing the M184V mutation in the absence of ZDV-associated mutations had the largest decline in HIV RNA among all genotypic groups (-0.97 $\log_{10}$ copies/mL DAVG$_{24}$). Patients with HIV expressing the high-level ZDV resistance mutation T215Y or F (47% of patients), NNRTI-associated, or PI-associated resistance mutations also responded significantly to tenofovir DF therapy. In addition, patients who expressed the less common nucleoside-associated RT mutations T69N/D, associated with zalcitabine and other nucleoside therapies, or L74V/I, associated with didanosine or abacavir therapy, also responded significantly to tenofovir DF therapy.

At baseline, five patients had HIV expressing the K65R RT mutation, a mutation associated with reduced susceptibility to tenofovir from *in vitro* studies, but also selected by abacavir and didanosine *in vivo*. These five patients were all randomly assigned to tenofovir DF therapy and did not respond to tenofovir DF therapy ($+0.12$ $\log_{10}$ copies/mL mean DAVG$_{24}$). Fewer patients assigned to tenofovir DF had HIV expressing mutations at the multinucleoside drug resistance site Q151M ($n = 2$) or the multinucleoside resistance insertion mutation after codon T69 ($n = 2$). Neither of these groups responded to tenofovir DF therapy with mean DAVG$_{24}$ values of $+0.38$ $\log_{10}$ copies/mL and $+0.29$ $\log_{10}$ copies/mL, respectively. The small numbers of patients and the lack of placebo controls in these genotypic groups, however, preclude conclusive determination of the effects of these mutations on response to therapy with tenofovir DF.

4.2.3.2.   Development of Mutations

Post-baseline genotypic data (week 24 or early termination) were obtained from 171 of 274 patients in the genotypic analyses substudy, with the remaining patients having insufficient HIV RNA to genotype ($n = 102$) or no post-baseline plasma sample ($n = 1$). Proportionally fewer patients in the tenofovir DF treatment arm (54%) than in the placebo arm (80%) had week 24 genotypic results due to a greater number of tenofovir DF treated patients who had insufficient HIV RNA for analysis.

Forty-seven patients developed one or more RT mutations at known nucleoside-associated resistance sites during the first 24 weeks (Table 4-20). Slightly fewer patients in the tenofovir DF treatment arm than in the placebo arm developed nucleoside-associated RT mutations (15% vs. 22%, respectively, $p = 0.17$, Fisher's exact test). Development of NNRTI- associated mutations were less common, but also occurred less frequently in the tenofovir DF arm (5% vs. 9%, $p = 0.17$). Significantly fewer patients developed PI-associated mutations in the tenofovir DF arm than in the placebo arm (2% vs. 8%, respectively, $p = 0.02$, Fisher's exact test).[92] Thus, it appears that tenofovir DF therapy was contributing to the suppression of nucleoside-, NNRTI- and PI-associated mutation development, consistent with significant decreases in viral load observed among tenofovir DF-treated patients.

Table 4-20.   **Development of Antiretroviral-Associated HIV Mutations by Week 24 in Study 907 (Genotyping Substudy, N = 274)**

RT and Protease Resistance Mutations Developing	Concomitant Nucleoside ART	Percent of Patients (n)		
		Placebo (N = 91)	Tenofovir DF (N = 183)	Total (N = 274)
Nucleoside-Associated		22% (20)	15% (27)	17% (47)
Any ZDV-Associated[1]		13% (12)	10% (19)	11% (31)[2]
M41L	d4T, ZDV, ddI, ABC, 3TC	3% (3)	4% (8)	4% (11)
K70R/Q/N	d4T, ZDV, ddI, 3TC	3% (3)	3% (6)	3% (9)
D67N	d4T, ZDV, ddI, 3TC	1% (1)	4% (7)	3% (8)
T215Y/F/I	d4T, ZDV, ddI, ABC, 3TC	5% (5)	2% (3)	3% (8)
L74V/I	d4T, ddI, ABC, 3TC	5% (5)	0.5% (1)	2% (6)
K65R	d4T, ZDV, ddI, ABC, 3TC		3% (5)	2% (5)
K219E/Q/R	d4T, ZDV, ddI, ABC, 3TC	2% (2)	1% (2)	1% (4)
L210W/S	d4T, ddI	1% (1)	1% (2)	1% (3)
M184V	ZDV, ABC, 3TC	2% (2)		1% (2)
T69N/I	d4T, ddI	1% (1)	0.5% (1)	1% (2)
V75I/A	d4T, ddI, ABC	1% (1)	0.5% (1)	1% (2)
A62V	ZDV, 3TC	1% (1)		0.4% (1)
Y115F	d4T, ABC, 3TC		0.5% (1)	0.4% (1)
Q151M	d4T, ABC, 3TC		0.5% (1)	0.4% (1)
Primary NNRTI-Associated[3]		9% (8)	5% (9)	6% (17)[4]
Primary PI-Associated[5]		8% (7)	2% (3)	4% (10)[6]

1   Zidovudine resistance mutations are M41L, D67N, K70R, L210W, T215Y/F or K219Q/E/N in RT.
2   22 of these patients also had ZDV-associated mutations at codons 41, 67, 70, 210, 215, or 219 at baseline (10 placebo, 12 TDF).
3   NNRTI resistance mutations are K103N, V106A, V108I, Y181C/I, Y188C/L/H, G190A/S/E, or P236L in RT.
4   11 of these patients also had primary NNRTI-associated resistance mutations at baseline (6 placebo, 5 TDF).
5   Protease inhibitor resistance mutations are D30N, V32I, G48V, I50V, V82A/F/T/S, I84V, or L90M in protease.
6   7 of these patients also had primary PI-associated resistance mutations at baseline (6 placebo, 1 TDF).
Data from reference: [92]

Development of Nucleoside-Associated RT Mutations

The majority of the patients (31 of 47) who developed nucleoside-associated mutations developed typical ZDV-associated mutations while taking zidovudine, stavudine, abacavir, or didanosine concomitantly. The capacity of stavudine, abacavir and didanosine to also select for "zidovudine-associated" mutations in vivo has been established.[84-88] There were no significant differences in the development of any of the ZDV-associated RT mutations between patients in the placebo and tenofovir DF arms of the study. Development of the D67N mutation appeared to occur more frequently in the tenofovir DF arm, but this was also not statistically significant (p = 0.28, Fisher's exact test). Among the seven patients who developed a D67N mutation, there was continued viral load suppression (-0.94 $\log_{10}$ copies/mL $DAVG_{24}$). Overall, the concomitant use of antiretroviral agents known to select for ZDV-associated mutations and their similar distribution between the treatment arms suggests that the concomitant antiretroviral agents were primarily responsible for their development.

Patients who developed nucleoside-associated RT mutations in the tenofovir DF treatment group showed continued viral load suppression in HIV RNA at week 24 (-0.51 $\log_{10}$ copies/mL $DAVG_{24}$, n = 27) similar to the -0.60 $\log_{10}$ copies/mL decrease in $DAVG_{24}$ observed for all patients treated with tenofovir DF in the virology substudy. Moreover, using the secondary endpoint of absolute change in HIV RNA from baseline, tenofovir DF treated patients who developed nucleoside-associated RT mutations during the first 24 weeks still showed a statistically significant mean HIV RNA decrease of -0.41 $\log_{10}$ copies/mL through week 24, suggesting continued anti-HIV activity despite the development of these mutations.[92]

Development of K65R RT Mutations

Five patients (2% of all patients, 3% of tenofovir DF treated patients) developed the K65R mutation, an RT mutation associated with zalcitabine, didanosine and abacavir in vivo, and also selected by tenofovir in vitro.[75, 89-91] All five patients were in the tenofovir DF treatment arm. Two of these patients were taking either didanosine or abacavir concomitantly, and three were taking lamivudine concomitantly along with tenofovir DF. Among these five patients, there was a notable variation in their response to tenofovir DF therapy, with a mean $DAVG_{24}$ of -0.29 $\log_{10}$ copies/mL (range: -1.10 to +0.72). Overall, few patients developed the K65R mutation and there was no consistent pattern of HIV RNA increases observed coincident with its development that would reflect treatment failure.

Development of Other Nucleoside-Associated RT Mutations

Fifteen patients developed one or more RT mutations at the other nucleoside-associated resistance codons (62, 69, 74, 75, 115, 151 or 184), and the emergence of substitutions at these codons was correlated with use of concomitant nucleoside analogs previously shown to select for these mutations (didanosine, stavudine, abacavir, zidovudine and lamivudine). The concomitant antiretroviral therapies that patients were taking are listed in Table 4-20. At

each of these RT residues, a new mutation developed in less than 3% of patients and there was a similar distribution between the tenofovir DF and placebo treatment arms. A single patient developed a mutation associated with multinucleoside drug resistance (Q151M) while taking tenofovir DF, but also stavudine and abacavir concomitantly. The capacity of stavudine to potentially select for the Q151M multinucleoside resistance complex has been described.[86-88] No patient developed an insertion mutation near codon T69 in plasma HIV during this study.

4.2.3.3.   Baseline Phenotypic Analyses

Baseline phenotypic analyses were attempted for all patients randomly assigned into the virology phenotyping substudy (n = 137) with the Virco Antivirogram[TM] assay; successful phenotypic results were generated for 85 of these patients (56 tenofovir DF, 29 placebo). Among these 85 patients, the mean number of ZDV-associated resistance mutations was 2.1 and the mean number of NRTI-associated resistance mutations was 3.2. Overall, the mean baseline susceptibility was 1.7-fold above wild-type control for tenofovir versus 7.6-fold above wild-type for ZDV and > 31.8-fold above wild-type for lamivudine. There were a total of nine patients who had HIV with > 4-fold reduced susceptibility to tenofovir. None of these nine patients had the K65R mutation at baseline, but had multiple nucleoside-associated mutations (mean = 4.8). No patient had HIV with > 10-fold reduced susceptibility to tenofovir at baseline as compared to wild-type HIV.

The HIV RNA response among various strata of baseline susceptibility to tenofovir is shown in Table 4-21. In intent-to-treat analyses, patients with baseline tenofovir susceptibility within 3-fold of wild-type responded with -0.42 to -0.72 $\log_{10}$ copies/mL decreases in HIV RNA through week 24. There were few patients within the other phenotypic strata, but there appeared to be a reduction in response consistent with the linear regression modeling. There were only five patients in the tenofovir DF arm with > 4-fold reduced susceptibility to tenofovir at baseline and, as a group, these patients did not appear to respond to tenofovir DF therapy.

Table 4-21.   **Response to Tenofovir DF Therapy by Baseline Tenofovir Susceptibility in Study 907**

Baseline Tenofovir Susceptibility (fold change from wild-type)	Tenofovir DF Mean $DAVG_{24}$[1] (n)		Placebo Mean $DAVG_{24}$[1] (n)	
	Intent-to-Treat	As-Treated	Intent-to-Treat	As-Treated
≤ 1.0	-0.72 (25)	-0.74 (25)	-0.05 (13)	-0.04 (13)
> 1.0 and ≤ 2.0	-0.50 (17)	-0.50 (17)	+0.03 (10)	+0.03 (10)
> 2.0 and ≤ 3.0	-0.42 (6)	-0.36 (6)	+0.41 (2)	+0.41 (2)
> 3.0 and ≤ 4.0	-0.27 (3)	-0.27 (3)		
> 4.0	-0.08 (5)	-0.08 (5)	-0.22 (4)	-0.22 (4)
All Patients Analyzed	-0.54 (56)	-0.54 (56)	-0.02 (29)	-0.01 (29)

1   Mean $DAVG_{24}$ for all patients in group ($\log_{10}$ copies/mL).
Data from reference: [92]

4.2.4.   Cross-Study Virology Analyses

Further evaluation of the antiviral response in patients with specific thymidine analog mutations (M41L, D67N, K70R, L210W, T215 Y/F, and K219Q) was undertaken using combined data from studies 902 and 907. In these analyses, the presence of either M41L or L210W mutation in combination with 3 other thymidine analog mutations predicted a reduced response to therapy.

4.2.5.   Virology Conclusions

In conjunction with the clinical trials, virology substudies were conducted in over 400 patients treated with tenofovir DF and the results support the in vitro and preclinical studies. In an integrated analysis of these virology substudies, the following conclusions can be made:

- Tenofovir DF once daily showed significant and durable HIV RNA reductions in patients with HIV expressing:

  - Zidovudine/thymidine analog resistance mutations

  - The M184V lamivudine resistance mutation

  - The T215Y high-level zidovudine resistance mutation

- · NNRTI- and PI-associated resistance mutations

- · Combinations of these resistance mutations

- Baseline phenotypic analyses demonstrated that:

  - · 90% of antiretroviral-experienced patients had HIV with susceptibility to tenofovir within 4-fold of wild-type and these patients responded to tenofovir DF treatment.

  - · Patients with up to 10-fold zidovudine resistance and > 30-fold lamivudine resistance responded to tenofovir DF therapy. A subgroup of patients with > 10-fold zidovudine resistance showed diminished responses to tenofovir DF therapy.

  - · Diminished responses to tenofovir DF therapy were observed in patients with > 4-fold reduced susceptibility to tenofovir at baseline. These patients had either the K65R RT mutation, a T69S double insertion mutation, or the T215Y/F and multiple other RT resistance mutations (mean 4.8) in their baseline HIV.

- Treatment of HIV-infected patients with tenofovir DF for up to 48 weeks is associated with infrequent development of resistance to tenofovir DF:

  - · Nine patients developed the K65R RT mutation (2.4% of treated patients).

  - · Development was not associated with an increase in plasma HIV RNA.

  - · No evidence for the development of novel resistance mutations from genotypic or phenotypic analyses was observed.

- Treatment with tenofovir DF was associated with a reduction in the development of NRTI-associated, or primary NNRTI-associated or PI-associated resistance mutations (study 907).

- Development of zidovudine/thymidine analog-associated mutations:

  - · Occurred similarly in the placebo and tenofovir DF arms.

  - · Appeared to be due to the patients' concomitant nucleoside therapy.

  - · Did not result in loss of viral load suppression.

- Reduced responses were noted in patients with either an M41L or L210W mutation in combination with 3 other thymidine analog mutations.

In summary, treatment with tenofovir DF results in:

- Significant antiviral efficacy through 24 - 48 weeks in highly antiretroviral-experienced patients with extensive nucleoside resistance in HIV at baseline.

- Low incidence of genotypic or phenotypic resistance to tenofovir arising during 24 - 48 weeks of tenofovir DF therapy.

- Reduction in the incidence of resistance mutations associated with nucleoside RT inhibitors, non-nucleoside RT inhibitors or protease inhibitors.

- Continued viral load suppression in patients who develop nucleoside-associated RT mutations.

4.3. Safety

Safety data from the Integrated Summary of Safety and from the Safety Update are summarized in this section. The Integrated Summary of Safety was included in the NDA submission (01 May 2001). The Safety Update was submitted to the FDA on 15 August 2001.

For the Integrated Summary of Safety data from studies 902 and 907 were pooled. A total of 149 patients in study 902 and 538 patients in study 907 received at least one dose of tenofovir DF 300 mg. As of 01 May 2001, 368 patients from study 907, 101 patients from study 902 and 6 patients from study 901 had rolled over to study 910. For the Safety Update, data from studies 902, 907, and rollover protocol 910 were pooled for the safety evaluation.

4.3.1. Treatment Duration (Exposure)

4.3.1.1. Pooled Studies (902, 907, 910)

In the pooled data from studies 902, 907, and 910, the mean duration of treatment in those patients who received at least one dose of tenofovir DF 300 mg is approximately 58 weeks (range: 0.4 to 143 weeks) as of the safety update data cut-off. Mean treatment duration described in the NDA was 36 weeks. See Table 4-22.

Table 4-22.   Treatment Duration (Pooled Studies)

	NDA			Safety Update
	Placebo (0-24 Weeks)	**TDF 300 mg (0-24 Weeks)**	**All TDF**	**All TDF**
Number of Patients	210	443	687	687
Weeks on Study Drug				
Mean ± SD	23.0 ± 4.0	23.0 ± 4.1	35.8 ± 30.4	58.2 ± 31.9
Median	24.0	24.0	28.1	50.1
Range	2.1 to 25.9	0.4 to 29.3	0.1 to 115.9	0.4 to 142.9

4.3.2.   Patient Disposition

4.3.2.1.   Pooled Studies (902, 907, 910)

A total of 687 patients received tenofovir DF 300 mg either initially or through rollover from another dose group. Figure 4-7 graphically presents the disposition of patients enrolled in studies 902 and 907. Most patients who completed studies 902 or 907 continued on tenofovir DF treatment in study 910.

Reasons for discontinuation for patients enrolled in studies 902, 907, and 910 are summarized in Table 4-23. The frequency of premature study discontinuation remains low despite prolonged treatment with tenofovir DF. In the All Tenofovir DF group, 29 patients (4%) discontinued tenofovir DF 300 mg due to adverse events or intercurrent illness, by the NDA data cutoff. As of the safety update cut-off, 44 patients (6%) had discontinued. Fifteen patients (2%) were lost to follow-up.

Figure 4-7.   **Patient Disposition - Safety Population (Patients Originally Enrolled in 902 and 907 Only)**
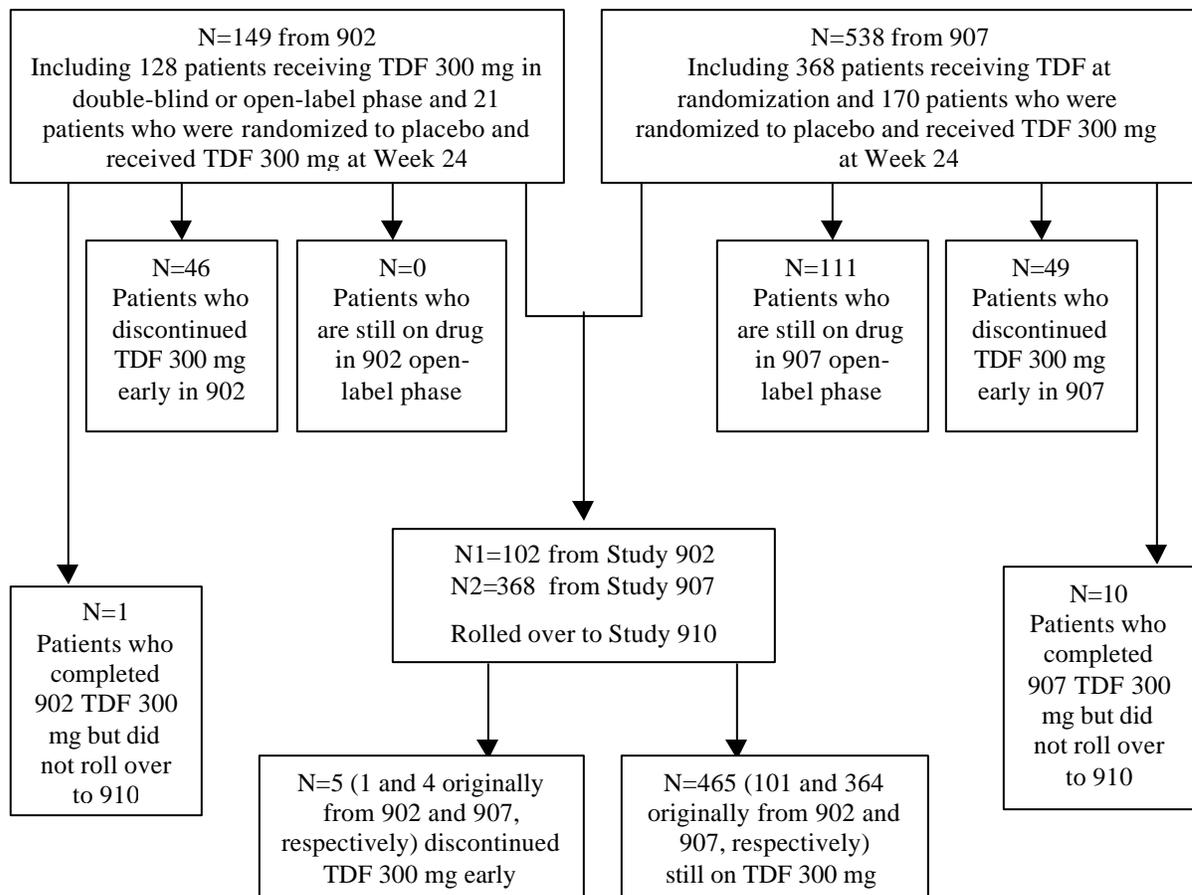
Note: The 6 patients from study 901 who rolled over into study 910 are not included.

Table 4-23.   **Disposition of Study Patients (Pooled Studies)**

	NDA						Safety Update	
	Placebo (0-24 Weeks) (N = 210)		TDF 300 mg (0-24 Weeks) (N = 443)		All TDF (N = 687)		All TDF (N = 687)	
Mean Duration (± SD) of Treatment (Weeks)	23.0 ± 4.0		23.0 ± 4.1		35.8 ± 30.4		58.2 ± 31.9	
Number of Patients	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Received at Least One Dose of Study Drug/TDF 300 mg	210	100%	443	100%	687	100%	687	100%
Discontinued Prematurely	18	9%	31	7%	70	10%	100	15%
Adverse Event	6	3%	13	3%	29	4%	44	6%
Lack of Virologic Response	3	1%	0	0%	3	<1%	7	1%
Lost to Follow-up	4	2%	7	2%	12	2%	15	2%
Non-compliance	0	0%	2	<1%	3	<1%	7	1%
Pregnancy	1	<1%	1	<1%	2	<1%	2	<1%
Other[1]	4	2%	8	2%	20	3%	25	4%

1   Other reasons for discontinuation include investigator decision, patient request, prohibited concomitant medications, or not specified.

4.3.3. Treatment Discontinuation for Adverse Events and Laboratory Abnormalities

4.3.3.1.   Pooled Studies (902, 907, 910)

Overall, the incidence of adverse events or laboratory abnormalities leading to discontinuation of tenofovir DF has remained low despite mean treatment durations of more than one year, and extending to nearly three years in some patients.

In the pooled studies, 35 patients (5%) who received at least one dose of tenofovir DF 300 mg discontinued study treatment due to adverse events. This is slightly higher than the 3% for this group reported in the NDA and the 2% reported for the 24-week placebo group (Table 4-24). No individual adverse event requiring study drug discontinuation occurred in more than 1% of patients. The most frequent event leading to discontinuation was nausea (7 patients, 1%). All other adverse events leading to study drug discontinuation occurred at frequencies less than 1%.

One percent of tenofovir DF-treated patients discontinued treatment due to laboratory abnormalities. Individual laboratory abnormalities that led to study treatment discontinuation occurred in less than 1% of patients (Table 4-25).

Table 4-24.   **Discontinuation Due to Adverse Events in Pooled Studies**

	NDA						Safety Update	
	Placebo (0-24 Weeks) (N = 210)		TDF 300 mg (0-24 Weeks) (N = 443)		All TDF (N = 687)		All TDF (N = 687)	
Mean Duration (± SD) of Treatment (Weeks)	23.0 ± 4.0		23.0 ± 4.1		35.8 ± 30.4		58.2 ± 31.9	
	n	%	n	%	n	%	n	%
Patients Who Discontinued Due to Adverse Events	**5**	**2%**	**11**	**2%**	**22**	**3%**	**35**	**5%**
Body as a Whole	**3**	**1%**	**6**	**1%**	**9**	**1%**	**15**	**2%**
Asthenia	1	<1%	3	<1%	5	<1%	6	<1%
Abdominal Pain	2	<1%	1	<1%	2	<1%	3	<1%
Malaise	0	0%	0	0%	0	0%	2	<1%
Pain	0	0%	1	<1%	1	<1%	2	<1%
Headache	1	<1%	0	0%	0	0%	1	<1%
Abdomen Enlarged	0	0%	0	0%	1	<1%	1	<1%
Hernia	0	0%	0	0%	0	0%	1	<1%
Carcinoma	0	0%	1	<1%	1	<1%	1	<1%
Digestive	**2**	**<1%**	**7**	**2%**	**8**	**1%**	**14**	**2%**
Nausea	2	<1%	4	<1%	5	<1%	7	1%
Anorexia	0	0%	0	0%	0	0%	2	<1%
Diarrhea	2	<1%	2	<1%	2	<1%	2	<1%
Vomiting	0	0%	1	<1%	1	<1%	2	<1%
Dysphagia	0	0%	0	0%	0	0%	1	<1%
Eructation	0	0%	1	<1%	1	<1%	1	<1%

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Table 4-24.   Discontinuation Due to Adverse Events in Pooled Studies (Continued)

	NDA						Safety Update	
	Placebo (0-24 Weeks) (N = 210)		TDF 300 mg (0-24 Weeks) (N = 443)		All TDF (N = 687)		All TDF (N=687)	
	n	%	n	%	n	%	n	%
Intestinal Obstruction	0	0%	0	0%	0	0%	1	<1%
Pancreatitis	1	<1%	1	<1%	1	<1%	1	<1%
Hematologic and Lymphatic	**0**	**0%**	**0**	**0%**	**1**	**<1%**	**1**	**<1%**
Thrombocytopenia	0	0%	0	0%	1	<1%	1	<1%
Metabolic and Nutritional	**2**	**<1%**	**0**	**0%**	**0**	**0%**	**6**	**<1%**
Dehydration	1	<1%	0	0%	0	0%	0	0%
Hyperlipidemia	1	<1%	0	0%	0	0%	0	0%
Lactic Acidosis	0	0%	0	0%	0	0%	1	<1%
SGOT Increased	0	0%	0	0%	1	<1%	1	<1%
SGPT Increased	0	0%	0	0%	1	<1%	1	<1%
Lipodystrophy	0	0%	0	0%	0	0%	1	<1%
Weight Loss	0	0%	0	0%	0	0%	3	<1%
Nervous System	**0**	**0%**	**2**	**<1%**	**6**	**<1%**	**11**	**2%**
Peripheral Neuritis	0	0%	0	0%	1	<1%	3	<1%
Depression	0	0%	0	0%	1	<1%	2	<1%
Paresthesia	0	0%	0	0%	1	<1%	2	<1%
Somnolence	0	0%	2	<1%	2	<1%	2	<1%
Dizziness	0	0%	0	0%	0	0%	1	<1%

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Table 4-24. **Discontinuation Due to Adverse Events in Pooled Studies (Continued)**

	NDA						Safety Update	
	Placebo (0-24 Weeks) (N = 210)		TDF 300 mg (0-24 Weeks) (N = 443)		All TDF (N = 687)		All TDF (N=687)	
	n	%	n	%	n	%	n	%
Encephalitis	0	0%	0	0%	1	<1%	1	<1%
Nervousness	0	0%	0	0%	0	0%	1	<1%
Respiratory	**0**	**0%**	**1**	**<1%**	**1**	**<1%**	**2**	**<1%**
Carcinoma of Lung	0	0%	1	<1%	1	<1%	1	<1%
Sputum Increased	0	0%	0	0%	0	0%	1	<1%
Voice Alteration	0	0%	0	0%	0	0%	1	<1%
Urogenital	**1**	**<1%**	**1**	**<1%**	**2**	**<1%**	**2**	**<1%**
Breast Enlargement	0	0%	0	0%	1	<1%	1	<1%
Gynecomastia	0	0%	0	0%	0	0%	1	<1%
Urinary Frequency	0	0%	1	<1%	1	<1%	1	<1%
Impotence	1	<1%	0	0%	0	0%	0	0%
Skin	**1**	**<1%**	**0**	**0%**	**0**	**0%**	**1**	**<1%**
Alopecia	0	0%	0	0%	0	0%	1	<1%
Seborrhea	0	0%	0	0%	0	0%	1	<1%
Rash	1	<1%	0	0%	0	0%	0	0%

Table 4-25. **Discontinuation Due to Laboratory Abnormalities in Pooled Studies**

	ISS						Safety Update	
	Placebo (0-24 Weeks) (N = 210)		TDF 300 mg (0-24 Weeks) (N = 443)		All TDF (N = 687)		All TDF (N = 687)	
Mean Duration (± SD) of Treatment (Weeks)	23.0 ± 4.0		23.0 ± 4.1		35.8 ± 30.4		58.2 ± 31.9	
Patients Who Discontinued Due to Laboratory	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Abnormalities	**4**	**2%**	**2**	**<1%**	**8**	**1%**	**12**	**1%**
Creatine Kinase Increased	2	<1%	1	<1%	4	<1%	4	<1%
AST Increased	0	0%	1	<1%	2	<1%	5	<1%
ALT Increased	0	0%	1	<1%	1	<1%	4	<1%
Triglycerides Increased	2	<1%	0	0%	2	<1%	2	<1%
Total Bilirubin	0	0%	0	0%	1	<1%	2	<1%
Serum Amylase Increased	0	0%	1	<1%	1	<1%	1	<1%
Lipase Increased	0	0%	1	<1%	1	<1%	1	<1%
Proteinuria	0	0%	0	0%	1	<1%	1	<1%
Hypokalemia	0	0%	0	0%	0	0%	1	<1%
Hyperkalemia	0	0%	0	0%	0	0%	1	<1%
Hypoglycemia	0	0%	0	0%	0	0%	1	<1%
Hyperglycemia	0	0%	0	0%	0	0%	1	<1%
Alkaline Phosphatase Increased	0	0%	0	0%	0	0%	1	<1%
Glycosuria	0	0%	0	0%	0	0%	1	<1%
Platelets Decreased	0	0%	0	0%	1	<1%	1	<1%

Note: Patients may have discontinued due to more than one event.

4.3.4.   Adverse Events

Table 4-26 below summarizes all adverse events reported in the pooled studies at a frequency of 5% or greater in either of the tenofovir treatment groups as of the NDA data cut-off.

Most patients experienced one or more adverse events. Comparing the placebo and tenofovir DF 300 mg 24-week treatment groups, a slightly higher incidence of gastrointestinal events (for example, diarrhea, nausea, and vomiting) was observed among tenofovir DF-treated patients. There was a statistically significant higher incidence of vomiting (12% vs. 6%, $p = 0.0225$) in the tenofovir DF 300 mg group compared with the placebo group. However, no adjustments for multiple comparisons were made. Importantly, < 1% of patients discontinued treatment with tenofovir DF due to these gastrointestinal events.

A significantly higher incidence of increased cough (9% vs. 4%, $p = 0.0226$) was reported in the tenofovir DF 300 mg group in the placebo-controlled periods of the pooled studies compared to placebo. Examination of the episodes of increased cough revealed that they occurred throughout the course of treatment in these studies and were uniformly associated with other symptoms of respiratory infection or seasonal allergic disorder. Therefore, it seems unlikely that increased cough would be attributed to treatment with tenofovir DF.

In the All Tenofovir DF group, which includes patients with longer durations of treatment, the most commonly reported adverse events were headache and non-specific systemic complaints such as asthenia, pain and viral infections, respiratory complaints of pharyngitis, rhinitis and sinusitis, gastrointestinal symptoms of nausea, diarrhea and vomiting, and rash. Importantly, the frequencies of adverse events in this group of patients were not notably higher than those observed during 24-weeks of treatment.

Table 4-26. **Adverse Events Reported in ≥ 5% of Tenofovir DF-Treated Patients in Pooled Studies – ISS**

	Placebo (0 – 24 Weeks)	TDF 300 mg (0 – 24 Weeks)	All TDF
Mean Duration (± SD) of Treatment (Weeks)	23.0 ± 4.0	23.0 ± 4.1	35.8 ± 30.4
Number of Patients Treated	210	443	687
Number of Patients Experiencing Adverse Events	187 (89%)	397 (90%)	523 (76%)
Body as a Whole			
Asthenia	35 (17%)	83 (19%)	127 (18%)
Headache	30 (14%)	61 (14%)	103 (15%)
Pain	34 (16%)	54 (12%)	99 (14%)
Viral Infection	19 (9%)	46 (10 %)	84 (12%)
Abdominal Pain	16 (8%)	43 (10%)	72 (10%)
Flu Syndrome	9 (4%)	21 (5%)	52 (8%)
Back Pain	13 (6%)	26 (6%)	50 (7%)
Fever	8 (4%)	21 (5%)	42 (6%)
Accidental Injury	7 (3%)	28 (6%)	46 (7%)
Digestive			
Diarrhea	36 (17%)	96 (22%)	154 (22%)
Nausea	32 (15 %)	89 (20%)	136 (20%)
Vomiting	12 (6%)	51 (12%)	81 (12%)
Dyspepsia	10 (5%)	26 (6%)	47 (7%)
Anorexia	8 (4%)	29 (7%)	45 (7%)
Flatulence	4 (2%)	27 (6%)	40 (6%)
Hematologic and Lymphatic			
Lymphadenopathy	4 (2%)	15 (3%)	31 (5%)
Metabolic and Nutritional			
Weight Loss	7 (3%)	19 (4%)	44 (6%)
Musculoskeletal			
Myalgia	16 (8%)	22 (5%)	37 (5%)
Nervous System			
Paresthesia	13 (6%)	24 (5%)	48 (7%)
Depression	14 (7%)	25 (6%)	47 (7%)
Insomnia	13 (6%)	29 (7%)	53 (8%)
Dizziness	14 (7%)	16 (4%)	31 (5%)
Anxiety	6 (3%)	16 (4%)	31 (5%)

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Table 4-26. **Adverse Events Reported in > 5% of TenofovirDF-Treated Patients in the Pooled 902 and 907 Studies (Continued)**

	Placebo (0 – 24 Weeks)	TDF 300 mg (0 – 24 Weeks)	All TDF
Respiratory			
Pharyngitis	32 (15%)	78 (18%)	144 (21%)
Sinusitis	23 (11%)	41 (9%)	89 (13%)
Rhinitis	18 (9%)	52 (12%)	91 (13%)
Bronchitis	6 (3%)	15 (3%)	31 (5%)
Cough Increased	8 (4%)	39 (9%)	67 (10%)
Skin			
Rash	17 (8%)	39 (9%)	72 (10%)
Sweating	5 (2%)	25 (6%)	45 (7%)
Herpes Simplex	7 (3%)	18 (4%)	33 (5%)

Note: A patient may be reported in more than one category.

4.3.5. Grade 3 or 4 Adverse Events

Grade 3 or 4 adverse events with a frequency of ≥ 1% are summarized in Table 4-27, as of the NDA data cut-off. The incidence of Grade 3 or 4 adverse events was low and similar for the placebo and tenofovir DF groups through 24 weeks. In the All Tenofovir DF group, frequencies did not increase compared to the 24-week data.

Table 4-27. **Grade 3 or 4 Adverse Events Reported in > 1% of Tenofovir DF-Treated Patients in the Pooled Studies**

	Placebo (0 – 24 Weeks)	TDF 300 mg (0 – 24 Weeks)	All TDF
Mean Duration (± SD) of Treatment (Weeks)	23.0 ± 4.0	23.0 ± 4.1	35.8 ± 30.4
Number of Patients Treated	210	443	687
Number of Patients Who Experienced Grade 3 or 4 Adverse Events	28 (13%)	62 (14%)	110 (16%)
Grade 3	21 (10%)	56 (13%)	92 (13%)
Grade 4	7 (3%)	6 (1%)	18 (3%)
Grade 3			
Abdominal pain	1 (< 1%)	3 (< 1%)	7 (1%)
Asthenia	2 (< 1%)	3 (< 1%)	7 (1%)
Depression	2 (< 1%)	3 (< 1%)	7 (1%)
Diarrhea	3 (1%)	4 (< 1%)	7 (1%)

Note: A patient may be reported in more than one category.

4.3.6. Marked Laboratory Abnormalities

Table 4-28 displays frequency of marked abnormalities for selected laboratory parameters among patients in the pooled studies. Marked laboratory abnormality is defined as a shift from grade 0 at baseline to at least grade 3 during study or from grade 1 at baseline to grade 4 during study.

Marked laboratory abnormalities occurred with similar frequencies in the placebo and the tenofovir DF groups during the 24-week blinded periods of the pooled studies and did not appear to increase with longer durations of treatment in the All Tenofovir DF group. The frequencies of marked changes of individual laboratory abnormalities were similar in all of the groups. Among all tenofovir DF-treated patients, 11 patients were identified who had marked changes in ALT, including three with chronic hepatitis B or C. Three patients had a concomitant increase in serum total bilirubin. All three of the bilirubin increases were Grade 1 in severity.

Elevations in ALT were significantly associated with the presence of underlying hepatitis. From the available data, there does not appear to be evidence of significant hepatotoxicity associated with tenofovir DF.

Table 4-28.   Selected Marked Laboratory Abnormalities (Pooled Studies)

	Placebo (0 – 24 Weeks)	TDF 300 mg (0 – 24 Weeks)	All TDF
Mean Duration (± SD) of Treatment (Weeks)	23.0 ± 4.0	23.0 ± 4.1	35.8 ± 30.4
Number of Patients Treated	210	443	687
Number of Patients Experiencing Marked Laboratory Toxicity by Highest Grade			
Grade 3	18 (9%)	41 (9%)	73 (11%)
Grade 4	13 (6%)	21 (5%)	42 (6%)
Hypertriglyceridemia			
Grade 0 → Grade 3	5 (2%)	6 (1%)	12 (2%)
Grade 0 → Grade 4	1 (< 1%)	3 (< 1%)	8 (1%)
Hypophosphatemia			
Grade 0 → Grade 3	1 (< 1%)	0 (0%)	1 (< 1%)
Grade 0 → Grade 4	0 (0%)	1 (< 1%)	1 (< 1%)
Creatine Kinase			
Grade 0 → Grade 3	2 (< 1%)	9 (2%)	26 (4%)
Grade 0 → Grade 4	6 (3%)	11 (2%)	20 (3%)
Grade 1 → Grade 4	3 (1%)	2 (< 1%)	3 (< 1%)

Table 4-28.   **Selected Marked Laboratory Abnormalities (Pooled Studies) (Continued)**

	Placebo (0 – 24 Weeks)	TDF 300 mg (0 – 24 Weeks)	All TDF
ALT			
Grade 0 → Grade 3	1 (< 1%)	5 (1%)	8 (1%)
Grade 0 → Grade 4	0 (0%)	1 (< 1%)	3 (< 1%)
Grade 1 → Grade 4	1 (< 1%)	0 (0%)	0 (0%)
AST			
Grade 0 → Grade 3	1 (< 1%)	9 (2%)	18 (3%)
Grade 0 → Grade 4	0 (0%)	1 (< 1%)	3 (< 1%)
Serum Amylase			
Grade 0 → Grade 3	4 (2%)	7 (2%)	13 (2%)
Grade 0 → Grade 4	0 (0%)	1 (< 1%)	1 (< 1%)
Glycosuria			
Grade 0 → Grade 3	4 (2%)	9 (2%)	13 (2%)

Note: Marked laboratory abnormality is defined as a shift from grade 0 at baseline to at least grade 3 during study or from grade 1 at baseline to grade 4 during study.

4.3.7.   Serious Adverse Events

4.3.7.1.   Pooled Studies (902, 907, 910)

During the first 24 weeks of treatment in the placebo-controlled studies 902 and 907, the incidence of serious adverse events was higher in the placebo-treated patients (8%) compared to those who received tenofovir DF (5%).

As of the data cut-off for the safety update, among all patients who received at least one dose of tenofovir DF 300 mg in these studies, the incidence of SAEs was 13%. Among these patients, only two events (pneumonia, 2%, and pancreatitis, 1%) occurred at frequencies ≥ 1%. All other events occurred in less than 1% of patients treated.

As noted in the NDA, the incidence of serious adverse events in the pooled studies judged to be possibly or probably related to tenofovir DF was low (less than 1%). As of the data cut-off for the safety update, one new possibly/probably related case (pancreatitis) had been reported.

During the 24-week placebo-controlled periods of studies 902 and 907, the incidence of serious adverse events was higher in placebo recipients compared to those patients who received tenofovir DF. With extended treatment duration, the incidence of serious adverse events increased somewhat, but individual events occurred infrequently, with only

pneumonia and pancreatitis occurring in 1% or more of patients. The cases of pneumonia were judged to be not related to tenofovir DF. Among all cases of pancreatitis in the pooled studies (7 patients, 1%), 2 cases were judged to be related to tenofovir DF. Serious adverse events judged related to study drug were uncommon, reported in less than 1% of treated patients.

Table 4-29.   **Serious Adverse Events in Pooled Studies (>1 Patient)**

Serious Adverse Events by Body System	Placebo (0-24 Weeks) (N = 210)		TDF 300 mg (0-24 Weeks) (N = 443)		All TDF (Safety Update) (N = 687)	
Mean Duration (± SD) of Treatment (Weeks)	23.0 ± 4.0		23.0 ± 4.1		58.2 ± 31.9	
Number of Patients Who Experienced Any Serious Adverse Event	**n**	**%**	**n**	**%**	**n**	**%**
	15	**7%**	**18**	**4%**	**89**	**13%**
Body as a Whole	**4**	**2%**	**4**	**<1%**	**22**	**3%**
Bacterial Infection	1	<1%	1	<1%	6	<1%
Cellulitis	1	<1%	2	<1%	3	<1%
Accidental Injury	0	0%	0	0%	3	<1%
Abscess	0	0%	1	<1%	2	<1%
Allergic Reaction	0	0%	1	<1%	2	<1%
Fever	1	<1%	0	0%	2	<1%
Abdominal Pain	0	0%	0	0%	2	<1%
Cardiovascular	**1**	**<1%**	**1**	**<1%**	**8**	**1%**
Digestive	**3**	**1%**	**6**	**1%**	**19**	**3%**
Pancreatitis	0	0%	2	<1%	7	1%
Colitis	1	<1%	0	0%	2	<1%
Nausea	0	0%	1	<1%	2	<1%
Diarrhea	0	0%	0	0%	2	<1%
Metabolic and Nutritional	**2**	**<1%**	**2**	**<1%**	**5**	**<1%**
Weight Loss	0	0%	0	0%	2	<1%

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Table 4-29. **Serious Adverse Events in Pooled Studies (>1 Patient) (Continued)**

Serious Adverse Events by Body System	Placebo (0-24 Weeks) (N = 210)		TDF 300 mg (0-24 Weeks) (N = 443)		All TDF (Safety Update) (N = 687)	
Number of Patients Who Experienced Any Serious Adverse Event	n	%	n	%	n	%
	17	8%	23	5%	89	13%
Musculoskeletal	**3**	**1%**	**1**	**<1%**	**12**	**2%**
Fracture	1	<1%	0	0%	6	<1%
Joint Disorder	2	<1%	0	0%	4	<1%
Nervous System	**2**	**<1%**	**1**	**<1%**	**14**	**2%**
Depression	1	<1%	1	<1%	3	<1%
Cerebrovascular Accident	0	0%	0	0%	2	<1%
Respiratory	**0**	**0%**	**3**	**<1%**	**20**	**3%**
Pneumonia	0	0%	2	<1%	12	2%
Lung Disorder	0	0%	1	<1%	3	<1%
Pneumothorax	0	0%	1	<1%	2	<1%
Asthma	0	0%	0	0%	2	<1%
Skin	**1**	**<1%**	**1**	**<1%**	**3**	**<1%**
Urogenital	**0**	**0%**	**1**	**<1%**	**11**	**2%**
Pyelonephritis	0	0%	1	<1%	3	<1%
Kidney Calculus	0	0%	0	0%	3	<1%
Urinary Tract Infection	0	0%	0	0%	2	<1%

4.3.8.   Targeted Evaluation of Renal and Bone Parameters

Based on preclinical studies, bone and kidney were identified as potential target organs for toxicity. Further evaluation of parameters specific to these two organs is provided in this section

4.3.8.1.   Serum Creatinine and Serum Phosphorus

Serum creatinine abnormalities were uncommon in the central laboratory database; no patient had a serum creatinine elevation above grade 1 (Table 4-30). Despite extended treatment duration, in the All Tenofovir DF group at the safety update data cut-off, 5% of patients had grade 1 creatinine elevations. Of the 32 patients with grade 1 creatinine elevations, 18 (3%) had serum creatinines $\geq$ 1.5 mg/dL, with a maximum of 1.9 mg/dL.

Fifteen percent of patients in the All Tenofovir DF group had grade 1 or 2 hypophosphatemia reported as of the safety update cut-off (Table 4-31). There were 4 patients who were reported to have grade 3 or 4 hypophosphatemia. One patient with grade 3 toxicity had this same grade at baseline, and the other three patients (two grade 3, one grade 4) had values within normal limits when the tests were repeated within 10 days of the abnormal finding. The lowest confirmed serum phosphorus value was 1.5 mg/dL.

To evaluate the largely sporadic and transient appearance of hypophosphatemia, an analysis was performed in patients who had $\geq$ grade 2 hypophosphatemia. Of the 62 patients (All TDF group) who had $\geq$ grade 2 hypophosphatemia, 51 had an abnormal value at only one visit, with the abnormality resolved by the subsequent visit. Ten patients had two consecutive grade 2 values; only one patient had 3 consecutive grade 2 values. No patient was discontinued from study due to hypophosphatemia.

With extended treatment duration, as of the data cut-off for the safety update, the incidence of serum creatinine elevations (5%) and hypophosphatemia (15%) in the pooled studies increased only slightly compared to the data presented in the NDA (4% and 14%, respectively). No patients had greater than grade 1 serum creatinine elevation or greater than grade 2 hypophosphatemia that was confirmed on retesting.

Serum creatinine elevations and serum phosphate decreases appear to occur sporadically in this patient population, do not worsen, and resolve with treatment interruption. To date, there remains no evidence of a significant tenofovir DF-related effect on these parameters.

Table 4-30.   **Maximum Grade of Laboratory Toxicity - Serum Creatinine (Pooled Studies)**

	NDA						Safety Update	
	Placebo (0-24 Weeks) (N = 210)		**TDF 300 mg (0-24 Weeks) (N = 443)**		**All TDF (N = 687)**		**All TDF (N = 687)**	
Mean Duration (± SD) of Treatment (Weeks)	23.0 ± 4.0		23.0 ± 4.1		35.8 ± 30.4		58.2 ± 31.9	
Maximum Grade of Laboratory Abnormality	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Grade 1 ($\geq$ 0.5 mg/dL over BL*)	3	1%	6	1%	25	4%	32	5%
Grade 2 (2.1 – 3.0 mg/dL)	0	0%	0	0%	0	0%	0	0%
Grade 3 (3.1 – 6.0 mg/dL)	0	0%	0	0%	0	0%	0	0%
Grade 4 (> 6.0 mg/dL)	0	0%	0	0%	0	0%	0	0%

Note: Grade was missing for one patient in the tenofovir DF 300 mg group.

* BL = Baseline

Table 4-31. **Maximum Grade of Laboratory Toxicity - Serum Phosphorus (Pooled Studies)**

	NDA						Safety Update	
	Placebo (0-24 Weeks) (N = 210)		TDF 300 mg (0-24 Weeks) (N = 443)		All TDF (N = 687)		All TDF (N = 687)	
Mean Duration (± SD)of Treatment (Weeks)	23.0 ± 4.0		23.0 ± 4.1		35.8 ± 30.4		58.2 ± 31.89	
Maximum Grade of Laboratory Abnormality	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Grade 1 (2.0 – 2.2 mg/dL)	10	5%	27	6%	43	7%	51	7%
Grade 2 (1.5 – 1.9 mg/dL)	5	2%	28	6%	48	7%	58	8%
Grade 3 (1.0 – 1.4 mg/dL)	1	<1%	0	0%	2	<1%	3	<1%
Grade 4 (< 1.0 mg/dL)	0	0%	1	<1%	1	<1%	1	<1%

Note: Grade was missing for one patient in the tenofovir DF 300 mg group.

4.3.8.2.   Bone Fractures

All adverse events and physical findings reported to the clinical databases for studies 901, 902, 903, 907, 908, and 910 were reviewed, evaluating all verbatim terms for evidence of bone fracture.

4.3.8.2.1.   Tenofovir DF Exposure

Table 4-32 details the exposure to tenofovir DF in studies 901, 902, 903, 907, and 908 based on the time of first dose to the last available date recorded on study, inclusive of central laboratory data. Studies 901 extension, 902, and 907 include the time on tenofovir DF for those patients who subsequently rolled over and are now being followed under study 910.

Table 4-32.   Tenofovir DF Exposure at Time of Data Summary

Study	Patients Who Received Tenofovir DF/Placebo	Total Exposure (Person-Years)
902 and 907 Placebo Group[1]	210	99
901, 902, 907 (combined)[2]	727	814
903[3]	600	388
908[4]	291	284

1   Includes 28 and 182 patients from studies 902 and 907, respectively.

2   Includes 10, 179, and 538 patients from studies 901, 902, and 907, respectively. Exposure in rollover study 910 is also included in the calculation.

3   Study is still in blinded phase; only overall data are available.

4   Five patients who never received study drug were excluded from the summary.

4.3.8.2.2.   Fracture Events

A total of 30 bone fractures, including eleven that were serious adverse events, are reported from the databases for studies 902, 903, 907, 908, and 910. Three of the thirty fractures were in patients receiving placebo in study 907. No fractures have been reported in the Expanded Access Program (study 955).

4.3.8.2.3.   Fracture Rates

Fracture rates have been calculated and are summarized in Table 4-33 for studies 903, 908, and the pooled data from studies 901, 902, and 907 as well as data from patients who rolled over into study 910. The fracture rate for the pooled data is presented by treatment group. The fracture rates in the tenofovir DF-treated patients are lower than the rate observed among

placebo-treated patients. All fractures were trauma-induced except for one patient with a history of osteoporotic fracture.

Table 4-33.   **Fracture Rates in Clinical Studies**

Study	Treatment Group	Number of Fractures	Fracture Rate[1] (95% CI)
902 and 907 Placebo Group[2]	Placebo	3	3.0 (0.6 – 8.9)
901, 902, 907 (combined)[3]	Tenofovir DF	16	2.0 (1.1 – 3.2)
903[4]	Tenofovir DF/ stavudine	4	1.0 (0.3 – 2.6)
908[5]	Tenofovir DF	7	2.3 (0.8 – 4.9)

1   Events/100 patient-years

2   Includes 28 and 182 patients from studies 902 and 907, respectively.

3   Includes 10, 179, and 538 patients from studies 901, 902, and 907, respectively. Exposure in rollover study 910 is also included in the calculation.

4   Study is still in blinded phase; only overall data are available.

7   Five patients who never received study drug were excluded from the summary.

4.3.9.   Study 908

Data from study 908, an open-label safety study that enrolled 296 patients with advanced AIDS (mean baseline CD4 = 36 cells/mL), also support the lack of significant toxicity attributable to tenofovir DF. The frequencies of grade 3 or 4 adverse events were somewhat greater in study 908 compared to the phase 2-3 studies, but the frequencies of individual serious adverse events was similar, and all of the reported deaths were attributed to HIV disease. The frequencies and severities of adverse events in this study are consistent with the advanced disease status of the enrolled patients.

4.3.10.   Safety Conclusions

The frequency of premature study drug discontinuation is low despite prolonged treatment with tenofovir DF. The incidence of discontinuations due to adverse events or intercurrent illness across the pooled studies is 6%. This rate compares favorably with discontinuation rates for studies of other antiretroviral therapies in similar treatment-experienced patient populations: 3% to 5% in a study of efavirenz and/or nelfinavir and 4% to 9% in a study of lopinavir/ritonavir and nevirapine.

Overall, the incidence of adverse events or laboratory abnormalities leading to discontinuation of tenofovir DF has remained low. In the pooled studies, the incidence has been 5% for adverse events and 1% for laboratory abnormalities.

The incidence of serum creatinine elevations (5%) and hypophosphatemia (15%) in the pooled studies each increased by 1% during additional follow-up since the NDA (from 4% and 14%, respectively). No patients had greater than grade 1 serum creatinine elevations and no patients had greater than grade 2 hypophosphatemia confirmed by a second consecutive measurement. The maximum observed peak serum creatinine in studies 902 and 907 was 1.9 mg/dL; nadir confirmed serum phosphorus was 1.4 mg/dL.

During the 24-week placebo-controlled periods of studies 902 and 907, the incidence of serious adverse events was higher in placebo recipients compared to patients who received tenofovir DF. Although the incidence of serious adverse events increased with extended treatment duration, individual events occurred infrequently - only pneumonia (2%) and pancreatitis (1%) occurred in 1% or more of patients. Related serious adverse events were uncommon, occurring in less than 1% of treated patients.

The incidence of bone fractures in study 908 and in pooled data from studies 902, 907, and 910 are 2.5 and 2.0 events/100 person-years of exposure, respectively. These rates are lower than that observed among patients treated with placebo for 24 weeks in studies 902 and 907 (3.0 events/100 person-years). Analysis of interval fracture rates showed sporadic increases and decreases in fracture rates over time, with no apparent trend. All fractures were trauma-induced except in the case of one patient with a history of osteoporotic fracture.

In conclusion, these safety data confirm that tenofovir DF has been well tolerated among treatment-experienced HIV-infected patients, for treatment durations as long as 143 weeks. There is no apparent evidence of significant drug-related toxicity associated with the use of tenofovir DF.

4.4.   Clinical Pharmacokinetics and Metabolism

The pharmacokinetic profile of tenofovir DF has been established following single and multiple dosing of intravenous tenofovir and oral tenofovir DF in HIV-1-infected patients and healthy subjects. The principal findings of the pharmacokinetic studies are:

- In HIV-infected patients, tenofovir pharmacokinetics were dose proportional over a dose range of 75 to 600 mg oral tenofovir DF and there was no clinically relevant change in the PK profile with daily dosing for periods of up to 24 weeks (studies 901 and 907).

- Tenofovir is primarily eliminated renally as unchanged drug with active tubular secretion by the kidney contributing to the elimination profile (studies 701, 901, and 914).

- The terminal half-life of tenofovir (approximately 11-14 hours) is sufficiently long enough to permit once daily dosing (studies 901 and 914).

- The oral bioavailability of tenofovir from tenofovir DF in fasted patients was approximately 25% (study 901). Administration of tenofovir DF with food (high-fat

meal), enhanced the oral bioavailability with an increase in tenofovir AUC by approximately 40% and C_{max} by approximately 14% (study 914).

- The pharmacokinetics of tenofovir were unaltered by concomitant administration with a variety of antiretroviral agents that are frequently taken in the HIV-infected population and are known to alter the pharmacokinetics of other medications (study 909). Although a higher serum concentration of didanosine was observed, data from the clinical studies provide no evidence that this interaction is of clinical significance. The slight decrease observed in the plasma concentration of ABT-378 is probably not clinically important.

4.4.1.   Tenofovir (IV)

In study 701, the pharmacokinetics of intravenous tenofovir were examined following single and repeated (7 days) administration at two dose levels (1.0 and 3.0 mg/kg) in a total of 16 patients with HIV infection.

Serum concentrations and $AUC_{0-\infty}$ increased in a dose-proportional manner following single intravenous doses of tenofovir. Concentrations of tenofovir in serum reached an observed maximum of 2.71 µg/mL and 8.52 µg/mL at the 1.0 and 3.0 mg/kg dose levels, respectively. Thereafter, serum concentrations declined in a bi-phasic manner, with an estimated mean terminal half-life of 5.3 hr and 7.8 hr for the 1.0 and 3.0 mg/kg dose levels, respectively. With the exception of the terminal half-life and volume of distribution, the pharmacokinetics of tenofovir were independent of dose over the dose range 1.0 to 3.0 mg/kg following the first dose. The mean renal clearance of tenofovir following the initial dose (~160 mL/hr/kg) exceeded creatinine clearance (~75 mL/hr/kg), indicating active tubular secretion contributes to the elimination profile of tenofovir. Approximately 70% to 80% of the administered tenofovir dose was excreted unchanged in urine within 72 hours following the first dose.

Following once daily dosing for 7 days at the 3.0 mg/kg/day dose level, there was an apparent decrease in serum and renal clearances of tenofovir. These observations may be due to inadequate assessment of the day 1 terminal phase of tenofovir.

4.4.2.   Tenofovir DF (Oral) (Studies 901 and 907)

The pharmacokinetics of oral tenofovir DF (75 mg, 150 mg, 300 mg, or 600 mg) and tenofovir DF 75 mg plus hydroxyurea (HU), following single and repeat daily dosing were evaluated in 46 HIV-infected adult patients in study 901. Subjects received a single dose of study drug in the fasted state and, after a seven-day washout period, 28 consecutive daily doses of study drug, each following a meal.

For the 75 mg and 150 mg dose cohorts, concentrations above the limit of quantitation of the bioanalytical assay were few, resulting in limited pharmacokinetic data. Median maximum serum tenofovir concentrations (C_{max}) were dose-proportional for the tenofovir DF 75, 150, 300, 600, and 75 mg + HU dosing cohorts at 68.6, 111, 240, 618, and 71.2 ng/mL, respectively. The time to reach maximum concentrations (T_{max}) was similar for all doses in

the fasted state (0.5-1.0 hours) and was delayed by 0.8 to 1.2 hours when administered with food. For the 300 mg and 600 mg dose groups, after C_{max} was achieved, serum tenofovir concentrations declined in a biexponential manner with median terminal half-life values of 11 and 13 hours, respectively, regardless of feeding state or pharmacokinetic visit.

Apparent clearance and renal clearance of tenofovir exceeded calculated creatinine clearance, indicating active tubular secretion of tenofovir by the kidney. Neither serum creatinine nor calculated creatinine clearance were affected by repeated administration (8 to 28 days) of tenofovir DF at any dose level.

Steady-state pharmacokinetic parameters evaluated on day 15 were dose-linear across all dose groups. There were no changes in pharmacokinetic parameters over time as assessed by comparison of total drug exposure following the first dose versus steady state. Tenofovir exposure over the dosing interval at steady-state for the 300 mg and 600 mg doses was similar to the total tenofovir exposure following the first dose.

Oral bioavailability of tenofovir was approximated using historical data obtained in an evaluation of 1 mg/kg intravenous dose. The bioavailability of tenofovir was enhanced by administration of food. Oral bioavailability of tenofovir DF 300 mg and 600 mg doses were estimated as 25% and 21%, respectively, in the fasted state, and as 39% and 34%, respectively, in the fed state. The median steady-state serum tenofovir exposure after 8 consecutive doses of tenofovir DF 150, 300, and 600 mg was 35%, 63%, and 131%, respectively, of that measured following administration of 1 mg/kg intravenous tenofovir.

The pharmacokinetics of tenofovir were also examined in 14 patients enrolled in the pharmacokinetic substudy of study 907. All pharmacokinetic assessments were performed in the fed state with the consumption of a standardized high-fat meal. The pharmacokinetics of tenofovir observed in this patient sample were comparable to those described for the shorter dosing period in study 901. Following the first oral dose of tenofovir DF the median C_{max} was 213 ng/mL and was achieved 2.4 hours following dosing. Thereafter, concentrations of tenofovir in serum declined in a bi-phasic manner with a median terminal half-life of 13 hours. The AUC_{0-8} on day 1 was 2750 ng*hr/mL.

As in study 901, the pharmacokinetics of tenofovir were not affected by repeat dosing. Steady-state pharmacokinetic parameters evaluated on week 12 (n = 7) were consistent with those predicted from day 1 results, indicating that the pharmacokinetics of tenofovir were not time-dependent. There was a small degree of tenofovir accumulation by week 12, consistent with the long serum half-life of tenofovir. Comparison of week 12 and 24 visits (n = 7) revealed no significant changes in tenofovir pharmacokinetics with time. In addition, calculated creatinine clearance was unchanged from day 1 through 12 and 24 weeks of study.

In summary, once daily oral administration of tenofovir DF 300 mg resulted in predictable serum tenofovir pharmacokinetics that were unchanged over 24 weeks of study.

4.4.3. Drug Interactions (Study 909)

Study 909 was a steady-state drug interaction study to assess the pharmacokinetic parameters of tenofovir DF (TDF) when administered alone or in combination with lamivudine (3TC), didanosine (ddI), indinavir (IDV), ABT-378/ritonavir and efavirenz (EFR) in healthy volunteers. For the purpose of examining pharmacokinetic parameters, healthy volunteers were selected for the study population to minimize the confounding effects of background antiretroviral and other therapies as well as the multiple, short-term changes in treatment regimens that may be required in HIV patients.

The study included five cohorts. In each cohort tenofovir DF was paired with either lamivudine, didanosine, indinavir, ABT-378/ritonavir, or efavirenz. All drugs were dosed to steady-state. Tenofovir DF, didanosine, lamivudine, and indinavir were administered for 7 days, while ABT-378/ritonavir and efavirenz were given for 14 days. When administered together with ABT-378/ritonavir or efavirenz, tenofovir DF was also given for 14 days. HIV medications chosen for study have the following pharmacokinetic characteristics:

- Lamivudine and didanosine are renally excreted and could compete with tenofovir for elimination.

- Indinavir inhibits the CYP450 enzyme system responsible for its own metabolism and other protease inhibitor metabolism, as well as the metabolism of many other drugs.

- ABT-378/ritonavir is a potent CYP450 inhibitor and induces the expression of CYP450 enzymes.

- Efavirenz is an inducer of CYP450 enzyme expression and inhibitor the metabolism of compounds metabolized by these enzymes.

Overall, the results of this study demonstrate a lack of clinically significant effects on the pharmacokinetics of tenofovir, when tenofovir DF was administered with other antiretroviral agents. Tenofovir DF had no significant effect on the pharmacokinetics of lamivudine, indinavir or efavirenz. The administration of ABT-378/ritonavir in combination with tenofovir DF caused a statistically significant increase (approximately 30%) in the C_{max} and $AUC_{(0-\tau)}$ of tenofovir; however, interpretation of this result is difficult. This may be a food effect rather than a drug-drug interaction. Administration of tenofovir DF with didanosine caused a 28% increase in the C_{max} and a 44% increase in the $AUC_{(0-\tau)}$ of didanosine. An analysis of patients using tenofovir and concomitant didanosine from the pooled dataset of 907 and 902 showed no evidence of an increased frequency of didanosine-related adverse events or laboratory abnormalities (pancreatitis, peripheral neuropathy, elevated amylase or lipase) when didanosine was administered with tenofovir DF. This suggests that the observed drug interaction is unlikely to be clinically significant.

Tenofovir DF also had slight but statistically significant effects on the disposition of ABT-378 causing an approximate 15% decrease in the C_{max} and $AUC_{(0-\tau)}$ parameters.

However, minimum plasma concentrations of ABT-378 were unaffected. This interaction is probably not clinically important.

4.4.4. Effect of Food (Study 914)

This study assessed the effect of food on the intended commercial formulation when administered in the fasted state. Following the first dose of tenofovir DF in fed patients, the mean concentration of tenofovir in serum was 335 ng/mL. The oral bioavailability of tenofovir from tenofovir DF in fasted patients was approximately 25%. Administration of tenofovir DF with food enhanced the oral bioavailability: geometric mean ratios for $AUC_{(0-\tau)}$ and $AUC_{0-\infty}$ were approximately 40% higher (140% and 138%, respectively), following administration with food. The 90% confidence intervals fell outside the 80% to 125% range, indicating a food effect. When tenofovir DF was administered in the fed state, the mean urinary excretion was higher (23.5%) compared with in the fasted state (16.9%), presumably due to the enhanced bioavailability. Mean renal clearance values were essentially identical in each treatment group. Renal clearance of tenofovir exceeded creatinine clearance indicating elimination via both active secretion and glomerular filtration. The terminal elimination half-life of tenofovir in serum averaged approximately 18 hours in each treatment group, supporting the once daily dosing schedule of tenofovir DF.

4.4.5. Demographic Effects on the Pharmacokinetics of Tenofovir

A rank ANOVA model that included factors for HIV-infection, gender, age, and weight was developed to assess the effects and/or potential relationships of demographic variables with the pharmacokinetics of tenofovir using pooled single-dose data from studies 901 (n = 8), 907 (n = 9), and 914 (n = 66).

There were no significant differences in the pharmacokinetics of tenofovir between HIV-infected patients (n = 17) and uninfected subjects (n = 36) (p > 0.1538) with the exception of terminal elimination half-life (p < 0.0001). This difference was likely due to a shorter duration of blood sampling post-dose in HIV-infected patients vs. healthy subjects (24 hours and 48 hours, respectively). There were no significant differences in the pharmacokinetics of tenofovir between females and males (p > 0.2559) or effect due to age (19 to 57 years) or body weight (p > 0.4305). There was insufficient data available from racial groups other than Caucasian to investigate potential pharmacokinetic differences among these populations. However, there do not appear to be substantial differences between races with regard to tenofovir pharmacokinetics.

There were no statistically significant differences in the pharmacokinetics of tenofovir due to gender, age, or body weight. There was insufficient data available from non-Caucasian patients to investigate potential pharmacokinetic differences among these populations; however, there do not appear to be substantial differences between races .

5.   PLANS FOR FURTHER DEVELOPMENT

A number of additional studies are underway or are planned as part of the tenofovir DF clinical development program.

5.1.   Study 903

A confirmatory double-blind active-controlled phase 3 study (study 903) designed to support traditional approval is ongoing, evaluating the safety and efficacy of tenofovir DF versus stavudine, both in combination with efavirenz and lamivudine in HIV-1-infected patients with plasma HIV-1 RNA levels > 5,000 copies/mL who are antiretroviral therapy-naïve. The enrolled patient population is 74% male with an average age of 35 years. Mean baseline HIV-1 RNA is 4.90 $\log_{10}$ copies/mL (range: 2.60 - 6.49), and mean baseline CD4 count is 277 cells/mm^3 (range: 3 - 1071). The study is fully accrued with 601 patients and 48-week data are expected to become available in early 2002. The study will continue blinded for 96 week, providing long-term, controlled safety and efficacy data.

5.2.   Study 928

Study 928 will be a second 48-week confirmatory study conducted in treatment-experienced, failing pediatric patients. The proposed design includes a blinded, placebo-controlled, 2-week evaluation of tenofovir DF as add-on therapy followed by optimization of background therapy and continuation of the blinded, placebo-controlled evaluation of tenofovir DF. A pediatric-appropriate formulation, currently in development, will be utilized in this study, scheduled to begin in March 2002.

5.3.   Study 910

As described previously, study 910 is a rollover protocol for patients who were receiving tenofovir DF in extended dosing phases of studies 901, 902, and 907. Study 910 will continue through December 2002, thereby permitting the continued collection of safety data from patients with the longest exposure to tenofovir DF.

5.4.   Study 917

A small, short-term (3-week) pilot study (study 917) to evaluate the viral dynamics of tenofovir DF 300 mg given once daily as monotherapy in a group of treatment-naive HIV-1-infected patients has been initiated. This study will provide additional data regarding the antiviral potency of tenofovir DF.

5.5. Expanded Access

Expanded Access Programs are underway in the U.S., Europe, Canada, and Australia. These programs are intended to provide tenofovir DF for patients with advanced HIV disease in need of a new antiretroviral agent and to collect long-term safety data in this population.

5.6. Collaborations

In addition to Gilead-sponsored studies, tenofovir DF 300 mg is included in the treatment regimens of a number of ongoing and planned collaborative studies. One, an Abbott-sponsored study conducted by Martin Markowitz, M.D. at the Aaron Diamond Center (M00-154; study 916), which began enrollment in late 2000, is an observational, 48-week, open-label study of complete viral suppression in antiretroviral-naïve patients. A second, a GlaxoSmithKline study (ESS 40006; study 918), which began enrollment in June 2000, is a randomized, open-label, 48-week study for patients with virologic evidence of treatment failure. Both of these studies will provide long-term efficacy and safety data. Several other collaborative efforts will help to further characterize the therapeutic utility of tenofovir DF in differenct segments of the HIV-infected population.

5.7. Other Studies

5.7.1. Pediatrics

In addition to study 928 (section 5.2), two phase 1 pediatrics studies are in development. studies 926 and 927 are 48-week open-label pharmacokinetic and safety studies, which target treatment-experienced children with advanced HIV disease.

Conducted at the National Cancer Institute, study 926 will enroll approximately 24 patients and will examine the single and multidose pharmacokinetics of tenofovir DF. In addition, during a 6-day monotherapy period, viral decay dynamics of tenofovir DF will be assessed. All patients will receive one of two doses of tenofovir DF as a component of a new antiretroviral regimen, which has been optimized using data gathered from resistance analyses. Besides evaluation of clinical adverse events and laboratory toxicities, bone metabolism will be measured using bone densitometry and specialized bone biomarkers of resorption and formation.

Study 927 will be conducted at the Necker Hospital in Paris, France, and will enroll approximately 20 patients. The single-dose pharmacokinetics of tenofovir DF will be examined. All patients will receive open-label tenofovir DF as a component of a new antiretroviral regimen, which has been optimized using data gathered from resistance analyses. Studies 926 and 927 are expected to begin enrollment in September 2001 and late 2001, respectively.

5.7.2. Pharmacokinetics - Renal Impairment

Since tenofovir is primarily eliminated unchanged by the kidney, a study of the pharmacokinetic disposition of tenofovir DF in patients with varying degrees of renal insufficiency is planned.

5.7.3. Pharmacokinetics - Hepatic Impairment

Although tenofovir is primarily eliminated by the kidney, a study of the pharmacokinetic disposition of tenofovir DF in patients with hepatic insufficiency is planned.

5.7.4. Drug Interactions

Studies to examine pharmacokinetic drug interactions between tenofovir DF and oral contraceptives, methadone, and enteric-coated didanosine are planned to begin in late 2001. In addition, a study to examine pharmacokinetic drug interactions between tenofovir DF and adefovir dipivoxil 10 mg (in development for the treatment of hepatitis B virus) will be performed.

6.   CONCLUSION

Tenofovir DF 300 mg once daily demonstrates statistically significant sustained antiviral activity in HIV-infected patients, including patients with genotypic evidence of nucleoside-resistant virus. To date, there is no evidence of the development clinical resistance to tenofovir DF. In addition, safety data confirm that tenofovir DF is well-tolerated by treatment-experienced HIV-infected patients, for treatment durations as long as 143 weeks. Tenofovir DF offers patients and clinicians a once daily, safe and efficacious agent for use in the treatment of HIV infection.

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8.   PRESENTATION SLIDES

Tenofovir Disoproxil Fumarate

NDA 21-356

October 3, 2001

Gilead Sciences, Inc
Foster City, CA

1

2

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Tenofovir Disoproxil Fumarate (TDF)

- **Overview of Development Program**
  Norbert Bischofberger, Ph.D.

- Clinical Trial Results
  Jay Toole, M.D., Ph.D.

- Phase IV Plans and Concluding Remarks
  Norbert Bischofberger, Ph.D.

Tenofovir Disoproxil Fumarate (TDF)
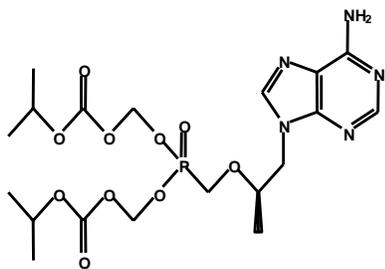
- Orally bioavailable prodrug of tenofovir (PMPA)

- Nucleotide RTI

- One pill once daily

- Activity against nucleoside resistant virus

Tenofovir DF: Pharmacology and Toxicology

- Efficacious in SIV models
- Long intracellular half-life (15-50 hr)
- Low potential for mitochondrial toxicity (based on in vitro data)
- Not a substrate, inhibitor, or inducer of CYP450 in vitro
- Excreted by kidney
- Main preclinical target organ toxicities:
  - Gastrointestinal - rat and dog
  - Kidney - dog and monkey
  - Bone - rat, dog and monkey

Tenofovir DF: Effects on Bone

- Tenofovir efficacy studies in newborn and juvenile monkeys (UC Davis)
  - Osteomalacia in animals dosed with tenofovir (sc) at 25x human exposure
  - Associated with hypophosphatemia, phosphaturia, ± glucosuria
  - Reversible upon dose reduction or cessation of treatment
  - No radiographic evidence of bone lesions in animals dosed at 8x human exposure for 3 yrs.

Tenofovir DF: Effects on Bone

- 10 month toxicology studies in rats and dogs
  - Marginal to slight reduction in bone mineral density (BMD) (pQCT) in animals dosed with TDF (p.o) at 6-10x human exposure
  - Associated with phosphaturia and calciuria
  - BMD reduction not progressive between 3 and 10 months of treatment
  - No BMD reduction in animals dosed at 2-3x human exposure
- Mechanism
  - Partial blockade of NaPi co-transporters in intestine (decreased PO_4 absorption) and kidney (decreased PO_4 reabsorption)

Tenofovir DF: In Vitro Virology

- Can select in vitro for the K65R mutation in RT

  - 3 to 4-fold reduced susceptibility to tenofovir

- Active in vitro against HIV with

  - ZDV resistance

  - ddI resistance (L74V)

  - ddC resistance (T69D)

  - multinucleoside drug resistance (Q151M)

- Increased activity against HIV with 3TC resistance (M184V)

Tenofovir Susceptibility of Nucleoside-Resistant HIV-1 Clinical Isolates

Virco Antivirogram™ Assay

	Mean Fold Change From Wild-Type Control	
M184V	0.7	(n = 10)
L74V	0.7	(n = 5)
L74V + Y115F + M184V	0.6	(n = 5)
T215Y (mean 3.3 TAMs)	3.1	(n = 20)
Q151M Complex	1.7	(n = 10)
K65R	2.5	(n = 8)
T69S Insertion	11.7	(n = 15)

Legend:
- Normal Range (≤ 3-fold)
- Intermediate Resistance (3-10-fold)
- Resistant (> 10-fold)

Axis: 0.1   1   3   10   100

Mean Fold Change From Wild-Type Control
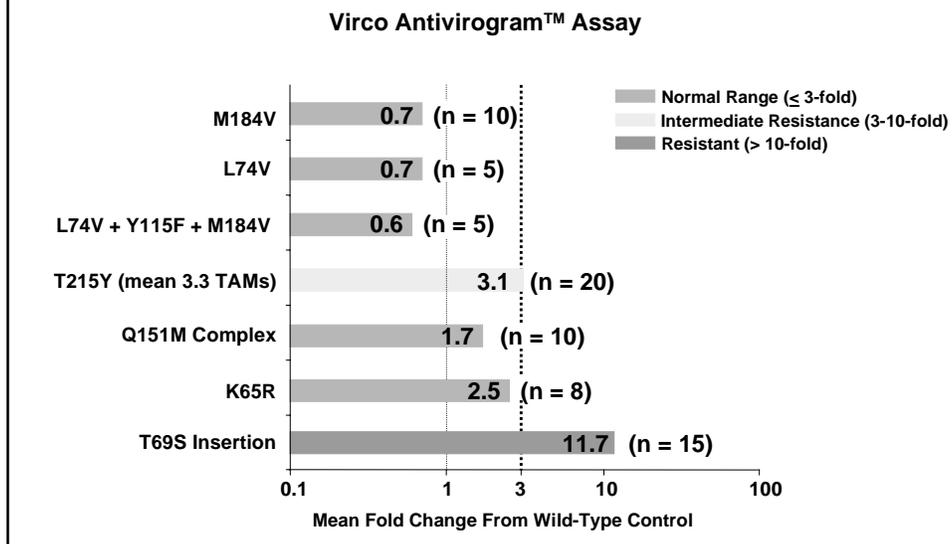
Tenofovir DF: Clinical Studies Submitted with NDA

- Placebo Controlled Clinical Studies
  - Study 901 Phase I/II   (n= 49)
  - Study 902 Phase II   (n=186)
  - Study 907 Phase III   (n=550)

- Study 908 (n=296)

  - Initiated December 1999 as compassionate access
  - Mean CD4: 36 cells/mL
  - Mean HIV-RNA: 4.9 $\log_{10}$ c/mL

- Drug Interaction (Study 914):
  - EVF, IDV, ddI, 3TC, LPV/RTV

- Food Effect (Study 909)

Tenofovir DF: NDA Safety Data Base

	Number of patients	
	Total	≥ 48 weeks exposure to TDF 300 mg
NDA Submission (May 1st)	978	185
NDA Safety update (August 15th)	978	792

Tenofovir Disoproxil Fumarate (TDF)

- Overview of Development Program
  Norbert Bischofberger, Ph.D.

- **Clinical Trial Results**
  Jay Toole, M.D., Ph.D.

- Phase IV Plans and Concluding Remarks
  Norbert Bischofberger, Ph.D.

Clinical Trial Results

- Placebo-controlled Studies

	Design	**Dose (qd)**
Study 901	Monotherapy (n=49)	75,150,300 & 600 mg
Study 902	Intensification (n=186)	75,150 & 300 mg
Study 907	Intensification (n=550)	300 mg

- Renal and Bone Parameters

Study 901
Design

- Randomized, double-blind, placebo-controlled, dose-escalation study of TDF monotherapy

  - HIV RNA $\geq$ 10,000 copies/mL; CD4 $\geq$ 200 cells/mm^3

  - 4 dose levels (75 mg, 150 mg, 300 mg, 600 mg/day)

- ~10 patients per dose level (8 active, 2 placebo)

- Single dose (day 1) followed by one week washout, then once-daily dosing (days 8 to 35)

- Treatment-naïve and experienced patients were enrolled

Study 901
Baseline HIV Characteristics

	Active (n=38)	Placebo (n=11)
Mean CD4 (cells/mm^3)	391	346
Mean HIV RNA (copies/mL)	85,351	115,593
Prior ART use	68%	36%

Study 901
HIV RNA

Mean Change from Baseline ($\log_{10}$ c/mL)
As Treated

	Placebo (n=9)	75 mg (n=10)	150 mg (n=8)	300 mg (n=6)	600 mg (n=8)
Day 35					
$\log_{10}$ c/mL	0.03	-0.33*	-0.51*	-1.20*	-0.84*

*p-values <0.003

Study 901
Mean Change from Baseline in HIV-1 RNA
Intent to Treat

17

Single Dose | Daily Dosing | Off Treatment

Mean Change (95% CI) $\log_{10}$ c/mL

DAYS OF STUDY: 4   8   14   21   28   35   42   63
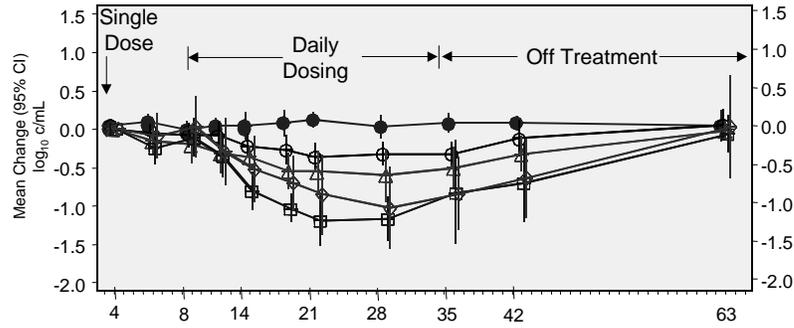
	n=							
● Placebo	11	9	8	9	8	9	9	8
○ TDF 75 mg	12	12	12	12	11	10	10	11
△ TDF 150 mg	8	7	8	8	8	8	8	8
□ TDF 300 mg	8	8	8	8	7	8	7	7
◇ TDF 600 mg	10	9	8	9	8	8	7	4

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Study 902
Design

- Randomized, double-blind placebo-controlled study of TDF added to existing antiretroviral regimen

- Entry criteria:

  - Stable ART $\geq$ 8 weeks prior to entry consisting of $\leq$ 4 concomitant antiretroviral agents

  - HIV RNA $\geq$ 400 - 100,000 copies/mL

- Primary Efficacy Endpoint

  - Time-weighted average change from baseline in HIV RNA ($\log_{10}$ c/mL) at week 24 (DAVG$_{24}$)

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Study 902
Design

	Double-blind		Open Label

	24 wks	48 wks

Stable ART ≥ 8 weeks randomized 2:2:2:1

300 mg	300 mg	300 mg
150 mg	150 mg	300 mg
75 mg	75 mg	300 mg
Placebo	300 mg	300 mg
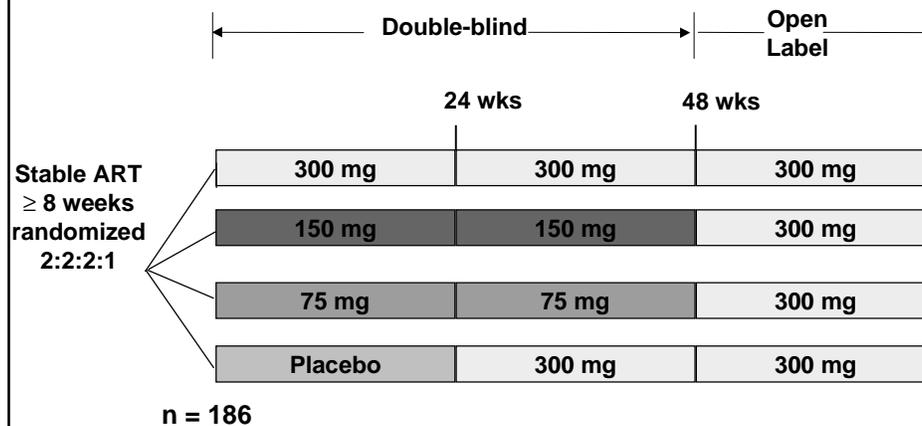
n = 186

Study 902
HIV Baseline Characteristics
(n=186)

- Mean CD4 (cells/mm^3)   374
- Mean HIV RNA (copies/mL)   16,583
- Mean prior ART (years)   4.6
- Baseline resistance
  - NNRTI   32%
  - PI   57%
  - NRTI   94%

Study 902
Patient Disposition
(0-24 weeks)

| | Placebo | TDF | | |
		75 mg	150 mg	300 mg
Patients who received drug	28	53	51	54
Patients discontinued (%)	7 (25%)	5 (9%)	8 (16%)	6 (11%)
Adverse events	1 (4%)	2 (4%)	5 (10%)	2 (4%)
Lost to follow up	2 (7%)	1 (2%)	2 (4%)	1 (2%)
Lack of virologic response	2 (7%)	0	0	0
Death	0	1 (2%)	0	0
Other	2 (7%)	1 (2%)	1 (2%)	3 (6%)

Study 902
Patient Disposition
(0-48 Weeks)

| | TDF | | |
	75 mg	150 mg	300 mg
Patients who received drug	53	51	54
Patients discontinued (%)	14 (26%)	12 (24%)	13 (24%)
Adverse events	6 (11%)	5 (10%)	5 (9%)
Lost to follow up	3 (6%)	5 (10%)	3 (6%)
Lack of virologic response	2 (4%)	0	0
Death	1 (2%)	0	0
Other	2 (4%)	2 (4%)	5 (9%)

Study 902
Grade 3/4 Adverse Events
(0-24 Weeks)*

	Placebo (n=28)	TDF 75 mg (n=53)	TDF 150 mg (n=51)	TDF 300 mg (n=54)
Patients (%) with Events	4 (14%)	10 (19%)	9 (18%)	9 (17%)
Depression	0	2 (4%)	0	3 (6%)
Asthenia	1 (4%)	0	2 (4%)	0
Hepatitis	1 (4%)	1 (2%)	0	0
Fever	1 (4%)	1 (2%)	0	0
Headache	1 (4%)	0	1 (2%)	0
Pancreatitis	1 (4%)	0	0	1 (2%)
Allergic Reaction	0	0	0	2 (4%)
Pain	0	1 (2%)	1 (2%)	0

*≥ 1% in either TDF or placebo

Study 902
Grade 3/4 Laboratory Abnormalities
(0 - 24 Weeks)*

	Placebo (n=28)	TDF 75 mg (n=53)	TDF 150 mg (n=51)	TDF 300 mg (n=54)
Patients (%) with Abnormality	9 (32%)	18 (34%)	16 (31%)	16 (30%)
Triglyceride elevation	4 (14%)	9 (17%)	4 (8%)	5 (9%)
Creatine kinase elevation	4 (14%)	5 (9%)	4 (8%)	6 (11%)
AST elevation	1 (4%)	3 (6%)	3 (6%)	4 (7%)
Neutropenia	1 (4%)	3 (6%)	1 (2%)	3 (6%)
ALT elevation	1 (4%)	2 (4%)	2 (4%)	1 (2%)
Lipase elevation	1 (4%)	1 (2%)	2 (4%)	1 (2%)
Amylase elevation	1 (4%)	2 (4%)	2 (4%)	0
Hyperglycemia	0	3 (6%)	2 (4%)	0
Glucosuria	0	2 (4%)	1 (2%)	0
Bilirubin elevation	0	1 (2%)	1 (2%)	1 (2%)
Thrombocytopenia	0	0	2 (4%)	0

*≥ 1% in either TDF or placebo

Study 902
Primary Efficacy Endpoint

Mean $DAVG_{24}$ ($\log_{10}$ c/mL)

	Placebo	TDF 75 mg	150 mg	300 mg
Intent to Treat	+0.02	-0.26	-0.34	-0.58*
As Treated	+0.16	-0.16	-0.32*	-0.52*

*p-value <0.001

Study 902
Mean Change from Baseline in HIV-1 RNA
Intent to Treat

n=											
● Placebo	28	28	27	27	26	26	22	23	0	0	0
■ 75 mg	53	48	49	53	49	48	49	48	49	46	42
♦ 150 mg	51	49	48	49	49	46	44	45	42	39	35
▲ 300 mg	54	52	51	52	52	50	49	48	49	43	43
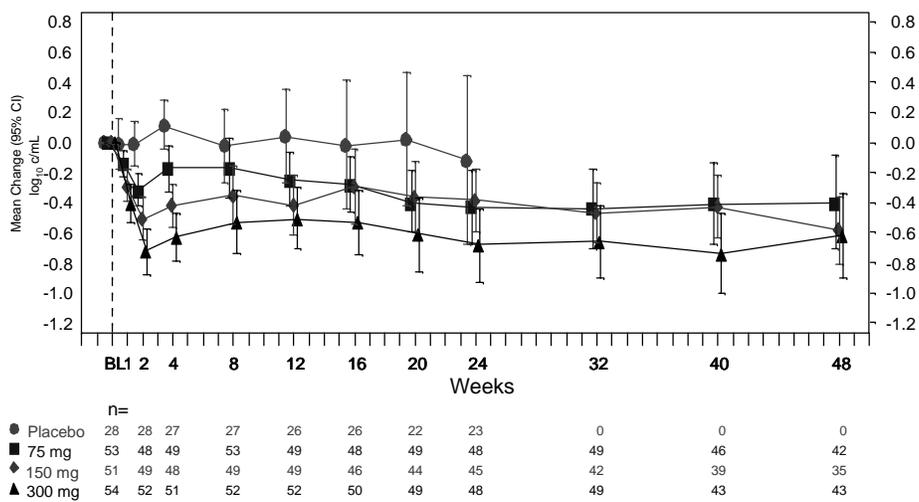
Study 902
CD4 Count

Mean Change From Baseline (ITT)

	Placebo (n=28)	TDF		
		75mg (n=53)	150mg (n=51)	300mg (n=54)
Week 24	20	18	0	-14
Week 48	N/A	10	20	11

Study 902
Virology
Response by Baseline Resistance Mutations
As Treated

Baseline Mutations	Mean DAVG$_{24}$ (log$_{10}$ c/mL)		p-value
	Placebo	TDF 300 mg	
M184V	+0.08 (16)	-0.64 (21)	<0.001
ZDV- R	+0.17 (20)	-0.52 (40)	<0.001
NNRTI - R	+0.25 (8)	-0.46 (17)	0.005
PI - R	+0.21 (18)	-0.61 (33)	<0.001

Study 907
Design

- Randomized, double-blind, placebo-controlled study of TDF added to existing antiretroviral regimen
- Entry criteria:
  - Stable ART $\geq$ 8 weeks prior to entry consisting of $\leq$ 4 concomitant antiretroviral agent
  - HIV RNA $\geq$ 400 - 10,000 copies/mL
- Primary efficacy endpoint
  - Time-weighted average change from baseline in HIV-1 RNA ($\log_{10}$ c/mL) at week 24 ($DAVG_{24}$)

Study 907
Design

$|\longleftarrow$ Double-blind $\longrightarrow|\longleftarrow$ Open Label $\longrightarrow|$

24 wks   48 wks

Tenofovir DF 300 mg

Stable ART $\geq$ 8 weeks randomized 2:1

24wks   48wks

Placebo   Tenofovir DF 300 mg

n=550
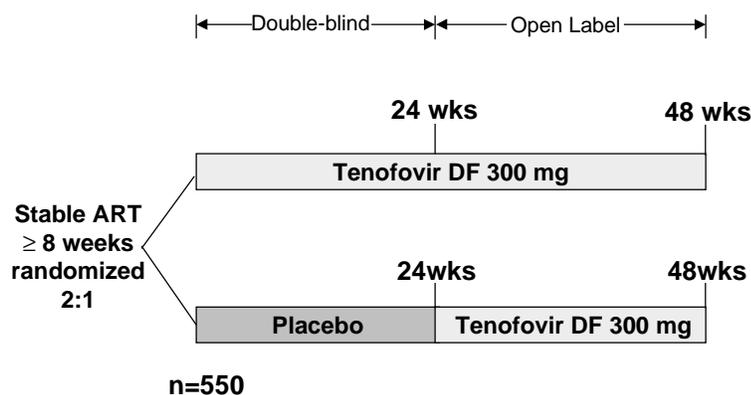
Study 907
Baseline Characteristics

	Placebo (n=182)	TDF (n=368)
Mean age (years)	41	42
Gender (M/F)	160/22	309/59
Ethnicity		
Caucasian	65%	71%
African-American	19%	16%
Other	16%	13%
Antiretroviral Regimen		
Protease-containing	58%	53%
NNRTI-containing	36%	43%

Study 907
HIV Characteristics

	Placebo	TDF
• Baseline Means		
– HIV RNA (copies/mL)	4365	4502
– CD4 count (cells/mm^3)	338	335
– ART use (years)	5.3	5.5

Study 907
Virology Substudy

Baseline Genotyping (n=253)

	Placebo	TDF
• Primary resistance mutations		
– NNRTI	52%	46%
– PI	62%	57%
– NRTI	94%	94%

Study 907
Patient Disposition
0-24 weeks

	Placebo		TDF	
Patients who received drug	182		368	
Patients discontinued (%)	11	(6%)	23	(6%)
Adverse event	5	(3%)	11	(3%)
Lack of virologic response	1	(<1%)	0	
Pregnancy	1	(<1%)	1	(<1%)
Lost to follow up	2	(1%)	6	(2%)
Other	2	(1%)	5	(1%)

Study 907
Grade 3/4 Adverse Events
(0-24 Weeks)*

	Placebo (n=182)	TDF (n=368)
Patients (%) with events	24 (13%)	51 (14%)
Diarrhea	3 (2%)	3 (<1%)
Pain	2 (1%)	3 (<1%)
Hyperlipidemia	2 (1%)	2 (<1%)
Nausea	2 (1%)	2 (<1%)
Depression	2 (1%)	1 (<1%)
Peripheral Neuritis	2 (1%)	1 (<1%)
Sinusitis	2 (1%)	1 (<1%)
Gastrointestinal Disorder	2 (1%)	0

*≥ 1% in either group

Study 907
Grade 3/4 Laboratory Abnormalities
(0-24 Weeks)*

	Placebo (n=182)	TDF (n=368)
Patients (%) with abnormality	68 (37%)	89 (25%)
Triglyceride elevation	24 (13%)	30 (8%)
Creatine kinase elevation	26 (15%)	24 (7%)
Amylase elevation	13 (7%)	21 (6%)
Glucosuria	6 (3%)	11 (3%)
AST elevation	5 (3%)	10 (3%)
Hyperglycemia	8 (4%)	7 (2%)
ALT elevation	3 (2%)	8 (2%)

*≥ 1% in either group

Study 907
Primary Efficacy Endpoint
Intent to Treat

	Placebo (n=182)	TDF (n=368)	p-value
Mean $DAVG_{24}$ ($\log_{10}$ c/mL)	-0.03	-0.61	<0.0001

Study 907
Mean Change from Baseline HIV-1 RNA
Intent to Treat
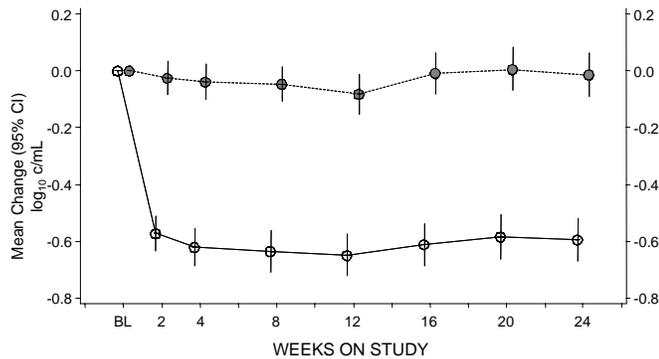
		BL	2	4	8	12	16	20	24
● PLACEBO	n= :	182	170	179	175	175	173	173	172
○ TDF 300 mg	n= :	368	335	358	353	354	353	346	346

Study 907
Subgroup Analyses

	Mean DAVG$_{24}$		
	Placebo	**TDF**	**p-value**
• HIV RNA < 5,000	0.03	-0.59	<0.0001
$\geq$ 5,000	-0.22	-0.67	<0.0001
• CD4 < 200	0.05	-0.39	0.0007
$\geq$ 200	-0.04	-0.64	<0.0001
• Male	-0.02	-0.61	<0.0001
Female	-0.08	-0.66	0.0002
• Caucasian	-0.02	-0.60	<0.0001
Non-caucasian	-0.05	-0.65	<0.0001

Study 907
Secondary Efficacy Endpoints
Intent to Treat

	Placebo	**TDF**	**p-value**
HIV RNA $\leq$ 400 copies/mL	13%	45%	<0.0001
HIV RNA $\leq$ 50 copies/mL	1%	22%	<0.0001
DAVG$_{24}$ CD4 (cells/mm^3)	-11	+13	0.0008

Study 907
Virology Substudy
Response by Baseline Resistance Mutations
As Treated

	Mean DAVG$_{24}$ (log$_{10}$ c/mL)	
	Placebo	TDF
Baseline Mutations	**(n=84)**	**(n=166)**
M184V	-0.04 (54)	-0.67 (117)*
ZDV- R	+0.03 (61)	-0.47 (113)*
NNRTI - R	+0.03 (44)	-0.48 (76)*
PI - R	+0.01 (52)	-0.55 (96)*

* p-values <0.0001

Study 907
Virology Substudy
Development of Resistance Mutations
(0-24 weeks)

	Placebo	TDF
Mutations	**(n=91)**	**(n=183)**
• NNRTI-related	9%	5%
• PI-related	8%	2%
• Nucleoside-related	22%	15%
– K65R	0	3%

Study 902 and 907
Renal and Bone Parameters
Integrated Safety Analysis

- Includes all patients who received 300 mg (n=687)

    - As randomized (n=422)

    - Following placebo cross-over (n=191)

    - Following 48 weeks of either 75 or 150 mg (n=74)

Studies 902 and 907
Integrated Safety Analysis*

- Number of patients   687
    - n ≥ 48 weeks exposure   480
    - n ≥ 72 weeks exposure   156
- Mean (weeks)   58
- Maximum (weeks)   143

*Through June 1, 2001

Study 907
Serum Creatinine
Maximum Toxicity Grade
(0-24 weeks)

Grade (mg/dL)	Placebo (n=182)	TDF (n=368)
1 ($\geq$ 0.5 from baseline)	2 (1%)	6 (2%)
2 (2.1 - 3.0)	0	0
3 (3.1 - 6.0)	0	0
4 (> 6.0)	0	0

Studies 902 & 907
Serum Creatinine
Maximum Toxicity Grade
(0-143 weeks)

Grade (mg/dL)	TDF (n=687)
1 ($\geq$ 0.5 from baseline)	32 (5%)
2 (2.1 - 3.0)	0
3 (3.1 - 6.0)	0
4 (> 6.0)	0

Studies 902 & 907
Creatinine Elevations are Transient
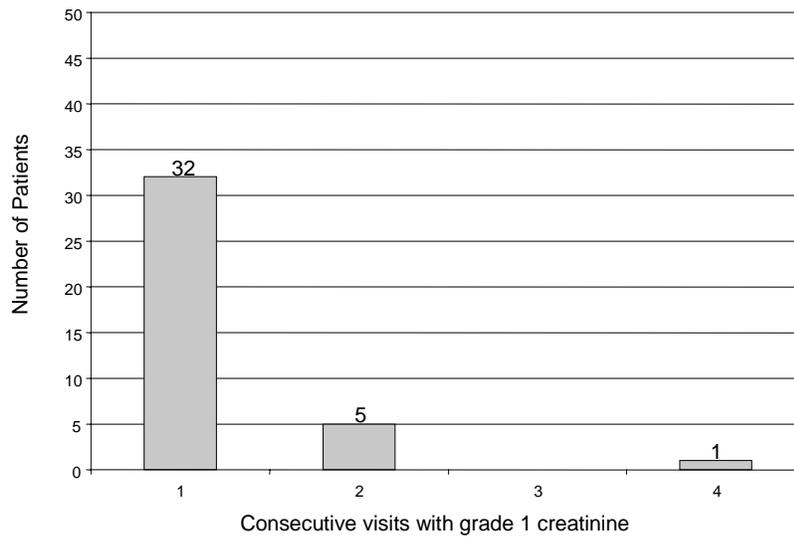
Study 907
Serum Phosphorus
Maximum Toxicity Grade
(0-24 weeks)

Grade (mg/dL)	Placebo (n=182)	TDF (n=368)
1 (2.0-2.2)	10 (5%)	21 (6%)
2 (1.5-1.9)	4 (2%)	23 (6%)
3 (1.0-1.4)	1 (<1%)	0
4 (<1.0)	0	1 (<1%)

Studies 902 & 907
Serum Phosphorus
Maximum Toxicity Grade
(0-143 weeks)

Grade (mg/dL)	TDF (n=687)
1 (2.0-2.2)	51 (7%)
2 (1.5-1.9)	58 (8%)
3 (1.0-1.4)	3 (<1%)
4 (<1.0)	1 (<1%)

Studies 902 & 907
Hypophosphatemia is Transient

Bar chart. Y-axis: Number of Patients (0 to 70). X-axis: Consecutive visits serum phosphate < 2.0 mg/dL. Bar 1: 62, Bar 2: 10, Bar 3: 1.
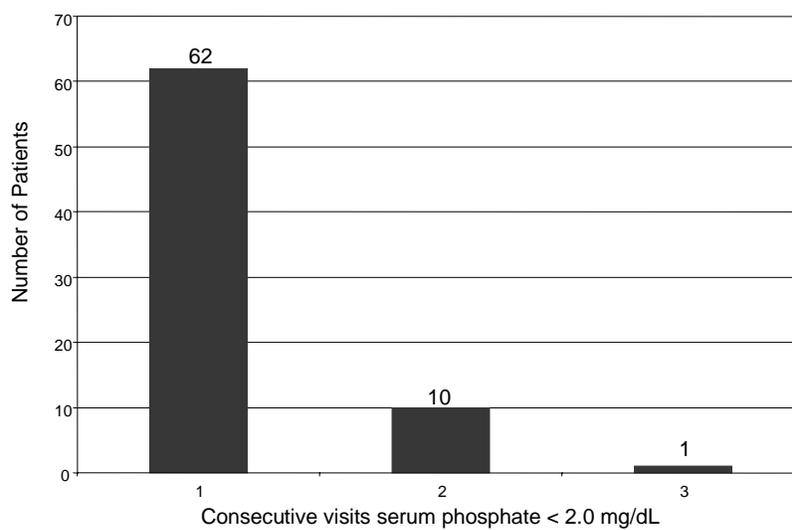
Studies 902 & 907
Bone Fracture Rate

	n	Total Exposure (patient-yrs)	No. Fractures	Fracture Rate* (95% CI)
Placebo (0-24 wks)	210	99	3	3.0 (0.6-8.9)
TDF (0-143 wks)	687	778	14	1.8 (0.9-3.0)

*Per 100 patient-years

Studies 902 and 907
Bone Fracture Summary

- External review of radiographs (H. Genant, M.D., UCSF)
- No vertebral compression fractures
  - Fractures result of high-impact trauma
  - Normal healing observed while TDF continued
- TDF event rate is similar to placebo
  - Rate has not increased with longer exposure

Tenofovir DF: Safety Summary

- The safety of TDF 300 mg is similar to placebo through 24 weeks

- The safety of TDF shows no significant change with extended dosing

Tenofovir DF: Efficacy Summary

- TDF 300 mg monotherapy resulted in -1.2 $\log_{10}$ c/mL change from baseline (901)

- TDF added to background regimens produced a significant reduction from baseline in HIV RNA of approximately 0.6 $\log_{10}$ c/mL in treatment-experienced patients (902 & 907)

  – durable through 48 weeks

Efficacy Summary
(Continued)

- TDF produced a significant increase in the percentage of patients with (907)
  - HIV RNA $\leq$ 400 copies/mL
  - HIV RNA $\leq$ 50 copies/mL
- Genotypic analyses demonstrate (902 & 907)
  - activity in patients with common HIV resistance mutations
  - low incidence of TDF resistance mutation development

Tenofovir DF: Clinical Conclusions

- TDF is safe and well-tolerated

- TDF provides durable antiviral suppression

- TDF has a favorable resistance profile

Tenofovir DF: Indication

Tenofovir DF is indicated in combination with other antiretroviral agents for the treatment of HIV infection in adults

Tenofovir Disoproxil Fumarate (TDF)

- Overview of Development Program
  Norbert Bischofberger, Ph.D.

- Clinical Trial Results
  Jay Toole, M.D., Ph.D.

- **Phase IV Plans and Concluding Remarks**
  Norbert Bischofberger, Ph.D.

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IIIb/IV Ongoing & Planned Studies

- Long term safety follow-up:

  – Study 910:   Open label rollover study from 902 and 907 (n=575)
  – Study 903:   Confirmatory study (n = 601)

- Pediatric development program:

  – Studies 926, 927:   Phase I/II
  – Study 928:   Phase III

- Expanded Access Programs:

  – Studies 950-955: US, Europe, Canada, Australia
  Enrollment (September 2001): 5000

Study 910

- Rollover protocol from studies 901, 902 and 907 (n=575)

- Continuing to evaluate patients through December 2002

  – Safety

  – Virology

  – BMD substudy (n = 87)

- Allows > 4 years of follow up for patients treated with TDF 300 mg

Confirmatory Study 903

- Design
  - Antiretroviral naïve patients
  - Randomization:

    $$1:1 \begin{cases} EFV + 3TC + D4T \\ EFV + 3TC + TDF \end{cases}$$

  - Blinded
- Enrollment completed 1/01; n = 601
- Bone evaluations in all patients:
  - BMD (DXA)
  - Bone biomarker (osteocalcin, bALP, N&C-teleopeptide, VitD, PTH)
- Endpoints
  - Efficacy: proportion of patients with HIV-RNA < 400 c/mL at week 48
  - Safety
- Duration of blinded study extended to 96 weeks

Study 903
Baseline Characteristics

- Female   26%
- Mean Age   35 years
- HIV-Related
  - Median HIV-RNA: 4.89 Log_{10} copies/mL
    Range: 2.6 - 6.49
  - Median CD4 Count: 262 cells/mL
    Range: 3 - 1071
  - % Symptomatic: 38%

Pediatric Development

- Pediatric development deferred pending safety evaluation in adults
- Pediatric formulation in development, available Q1 2002
- Phase I/II studies:
  - Study 926: Single/multiple dose PK
    n=10 (France); initiated 9/01
  - Study 927: 48 week, safety, efficacy
    n=   (NCI); initiated 10/01
- Phase III study:
  - 48 week placebo controlled study of TDF added to optimized background regimens
  - 2nd confirmatory study

Tenofovir DF: Conclusions

- Evidence for safety and efficacy from controlled clinical studies (901, 902, 907)

- Evidence of longer term safety (renal, bone) from safety update

- Long term safety and efficacy studies (903, 910) in progress

- Pediatric development and additional supportive studies initiated or planned